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Effect of fermentation conditions on alcohol production by yeasts during processing of mango wine

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Abstract: The present investigation was focused on the selection of desirable yeast for the production of high-quality wine from mango. In this study six wine yeasts were procured from different sources and evaluated for ethanol tolerance and effect of pH, temperature and sugar concentration on their fermentation efficiency. Among six wine yeasts tested for their sugar (25%) and ethanol (16%) tolerance, a strain of *Saccharomyces bayanus* was found to be better than the other 5 strains of *S. cerevisiae*. The strain *S. bayanus* produced more ethanol (10%) than the other strains under various conditions of pH and temperature in mango wine, the optima being 4.0 and 25°C, respectively. The physiochemical characteristics of wines produced by these strains, was un-equivalently found that *S. bayanus* is better in terms of desirable production of volatile acidity, higher alcohols, pH, and esters. Hence, the strain of *S. bayanus* has been selected as suitable yeast strain for further studies for production of mango wine.

Keywords: Alcohol, *Saccharomyces cerevisiae*, Mangowine, pH, Temperature

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Yeasts are the most predominant species from the ancient times in the complex process of wine making. Several researchers revealed the hidden world of microbial activity during wine fermentation and proved that yeast is the primary catalyst in wine fermentation. Yeasts and alcoholic fermentation have incited a considerable amount of research, making use of progress in enology and brewery science. Although many genera and species of yeast are found in the world, the genus *Saccharomyces*, and mainly the species *S. cerevisiae* are considered to be the most popular species in the fruit juice fermentations. Because of this, *S. cerevisiae* is referred as "the wine yeast" (Pretorius 2000).

The budding yeast *Saccharomyces cerevisiae* has a long history in the fermentation industry. Owing to its efficiency in producing alcohol, *S. cerevisiae* is the most important commercial microorganism with GRAS (Generally Regarded As Safe) status (Pretorius et al. 2003). Mankind's oldest domesticated organism is used for brewing beer and other alcoholic beverages. Though in our days the use of yeast extended in modern molecular genetics, ethanol produced by yeast fermentation still remains in the first place. Ethanol is an important industrial chemical with emerging potential as a biofuel to replace vanishing fossil fuels (Alfenore et al. 2002). So, the demand for yeast strains with a good fermentative efficiency and increased alcohol tolerance remains topical.

The selection of a good yeast strain having desirable properties is a prerequisite for the quality wine production (Degree 1993). Enological traits of *S. cerevisiae* have been divided into two groups, i.e. technological and quailtative, and both groups have to be considered in the selection of wine yeasts. The technological ones influence the fermentation efficiency, and the qualitative ones determine the chemical composition and sensorial characteristics of wines. Yeast should have a corresponding metabolic profile, i.e. it should be chosen according to aroma and flavor that is typical for each wine (Sudheer et al. 2012; Romano et al. 1998; Raineri and Pretorius 2000).

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The principal metabolic process in wine making is the alcoholic fermentation, which consists in the biotransformation of grape sugars (glucose and fructose) into ethanol and carbondioxide. The principal organism responsible for this transformation is the yeast. For high glucose concentrations (approximately above 9 g/L), *S. cerevisiae* only metabolizes sugars by the fermentative pathway. Even in the presence of oxygen, respiration is blocked. This phenomenon, firstly named as Crabtree effect, is also known as catabolic repression by glucose or the Pasteur contrary effect (Ribereau-Gayon et al. 2000). So, in *S. cerevisiae* glucose and fructose are metabolized to pyruvate via glycolysis pathway. Pyruvate is decarboxylated to acetaldehyde, which is then reduced to ethanol. During wine yeast glycolysis, one molecule of glucose or fructose yields two molecules of ethanol and carbon dioxide. However, the theoretical conversion of 180 g sugar into 92 g ethanol and 88 g carbondioxide could only be expected in the absence of any yeast growth, production of other metabolites and loss of ethanol as vapor. In a standard fermentation, about 95% of the sugar is converted into ethanol and carbon dioxide, 1% into the cellular material and 4% of other products such as glycerol.

This paper describes the effect of different conditions on the production of mango wine by yeasts. In this work, we have selected six different yeasts (for the fermentation of mango wine. The objective of the present study is to investigate the effect of fermen-tation conditions on mango wine production by using different yeast strains and to observe changes in physicochemical properties of mango wine.

MATERIALS AND METHODS

Materials

Alphonso variety mango pulp was obtained from the Mysore Fruit Products, Bangalore, India. Potential wine yeast strains were obtained from well-known laboratories. A commercial pectinase enzyme (Bio-Tropicase) was obtained from Biocon India Pvt. Ltd, Bangalore, India. Citric acid and $CaCO_3$ for pH adjustment, potassium metabisulphite for sulphation of juice, 3, 5-Dinitro Salicylic acid (DNS) reagent for sugar estimation, and n-propanol as an internal standard for gas chromatography were obtained from Sd-Fine Chemicals (India).

Three wine yeast strains procured from the National Chemical laboratory (NCL), Pune, India, namely *Saccharomyces cerevisiae var. ellipsoideus* NCIM 3215 (ATCC 4921), a French wine yeast, *S. cerevisiae* NCIM3189 (IISC.9) an Australian wine yeast No.117, and *S. cerevisiae* NCIM3045 (IFO 0209), Two other wine yeast strains (*Saccharomyces cerevisiae var. bayanus* and *Saccharomyces cerevisiae*I was obtained from the University of Turin, Italy as a complement. Another yeast strain obtained from CFTRI, Mysore, India (*Saccharomyces cerevisiae* CFTRI 101). All these 6 yeast strains were subcultured periodically and maintained on MPYD agar medium.

Mango juice preparation

The mango pulp was treated with 0.1% (w/v) of pectinase enzyme and incubated at 40 °C for 2 h. The activity of enzyme reaction was stopped by heating at 100 °C for 10 min. The juice was extracted by passing the treated pulp through cheesecloth and stored at 4 °C for further studies.

Inoculum preparation

The yeast cultures were maintained on MPYD agar (Himedia, India) medium containing (g/l): malt extract 3.0, peptone 5.0, yeast extract 3.0, dextrose 10.0, and agar 20.0, and subcultured every month on agar slants. The yeast cells from slant culture were inoculated into 25 ml of the sterile MPYD liquid medium in 100 ml Erlenmeyer conical flask, incubated on a rotary shaker (100 rpm) for 24 h. This growth (3×10^6 cells/ml) was transferred to 250 ml conical flask having 100 ml sterile mango juice and incubated at 28 °C, on a rotary shaker (100 rpm) for 24 h and was used as inoculum for wine fermentation.

Production media and fermentation conditions

The °Brix of the mango juice was determined by using a hand held refractometer (Erma, Japan). The final °Brix of the mango juice was adjusted to 20 °Brix and 100 ppm level of potassium metabisulphite was added in the juice to prevent the bacterial contaminants. The pH was set to the desired value by means of calcium carbonate or citric acid addition before inoculation. Erlenmeyer flasks (500 ml) dispensed each with 300 ml treated juice were inoculated with varied inoculum and fermented at stationary conditions at different pH, sugar concentration and temperatures for 10 days.

Ethanol tolerance of yeast strains

The YEPD medium without or with an appropriate ethanol concentration (6-20% v/v) was used for the screening of yeasts for ethanol tolerance. The medium was sterilized at 121°C for 15 min in an autoclave and cooled. Enough absolute ethanol was then added to different flask of the same medium to constitute varying percentages of ethanol differing by 2 % (v/v) from one flask to the other. The media were triplicated and inoculated separately with each of the yeast strain. The initial optical density of each flask was read off on a spectrophotometer (Elite, India) at 615 nm against the medium as blank. The inoculated flasks were transferred in a rotary shaker incubator set at 150 rpm at 30°C for 72 h. The increase in optical density in a flask was recorded as an evidence of growth. The concentration of alcohol at which the growth of the yeast was just inhibited was assessed as the ethanol tolerance of the yeast. Only the yeast strains that showed growth in 8% ethanol (v/v) were further examined.

Sugar tolerance of yeast strains

To study the effect of sugar concentration on yeast growth using the method of Ekunsanmi and Odunfa

(1990) was employed. The medium was sterilized by autoclaving at 121°C for 15 min, cooled and inoculated with 0.1 ml of cell suspension of each isolated strain. The turbidity was measured by spectrophotometer at 540 nm after inoculation and subsequently at intervals of 12 h over a period of 60 h. The YEPD medium used contained yeast extract, 10 g/l; peptone, 20 g/l, and glucose added in concentrations of 50, 100, 150, 200 and 250 g/l.

Determination of reducing sugars, glycerol, and volatile acidity

Total reducing sugars were determined spectrophotometrically using DNS method (Miller 1959). The analysis of glycerol present in the wine samples was performed enzymatically by the method of Wieland (1988), using commercially available glycerol assay kit. Volatile acidity was estimated according to the O.I.V method (Apetrei et al. 2007).

Estimation of alcohol by GC-FID

The estimation of ethanol was carried out by direct injection of distilled wine sample into gas chromatography (Neon pro, Ashco Ind. Ltd., India) using capillary column, BP20 (SGE, Australia; 30M × 0.25MM ID × 0.2 µm film thickness of polyethylene glycol). Nitrogen was used as a carrier gas with a flow rate of 20 ml/min. Fuel gas used in this process was hydrogen with a flow rate of 40 ml/min and the oxidant was air with a flow rate of 400 ml/min respectively and the eluted compounds were detected by flame ionization detector (FID). The detector and injector temperatures were maintained at 280 and 240°C, respectively while the oven temperature was maintained at a constant temperature of 145°C. A linear calibration curve was obtained by injecting wine sample along with n-propanol as an internal standard and calculating the area under the peak.

Results and Discussion

The yeast strain used during fermentation can have a great influence on the ultimate quality and quantity of the final product. So, the selection of the yeast strain is the crucial step for an expected and assured good quality wine. These yeast strains were subjected to screening for evaluating fermentation performance at different temperature and pH during fermentation. Of the six yeast strains *Saccharomyces cerevisiae var. bayanus*, *Saccharomyces cerevisiae* (Italian wine yeast) and *Saccharomyces cerevisiae* CFTRI 101 gave promising results in all conditions.

Effect of pH on alcohol production in mango wine

Ethanol production varied with the pH change of mango juice. The pH of the mango juice was adjusted in the range of 2.5 to 5.5 using citric acid (acidic pH) and $CaCO_3$ (basic pH). The lowest concentration of ethanol was observed at pH 2.5 and highest at pH 4 with all the strains (Fig. 1). The ethanol concentration was increased gradually with pH 2.5 to pH 4. Of all the strains, *Saccharomyces bayanus* has shown the highest ethanol production in mango wine at pH 4 followed by *S. cerevisiae* (Italian strain).

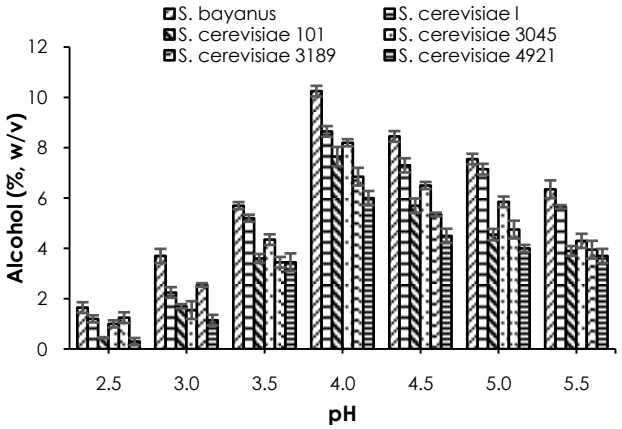

Figure 1: Effect of pH on alcohol (%) production in mango wine by six wine yeast strains

Although ethanol is the principal metabolite from glucose fermentation by *S. cerevisiae*, organic acids are also produced which lower the pH of the fermentation broth to as low as pH 3.5. Since the pH optima for glycolytic enzymes are at the near neutrality, the failure of *S. cerevisiae* to maintain large differences in pH during the accumulation of ethanol might be the cause of the rapid decline in the fermentative activities of cells despite the abundance of glycolytic and alcohologenic enzymes (Dombek and Ingram 1987).

Effect of temperature on alcohol production in mango wine

Temperature plays a vital role in the yeast growth and fermentation performance, as the yeast growth varies depending upon the degree of temperature. In the present study, six yeast strains were subjected to alcoholic fermentation at varied temperatures (10-35°C) to find out optimum temperature for maximum production of ethanol by a given strain during mango wine production. Fig. 2 shows that all the yeast strains produce high alcohol content at 25°C. The alcohol production gradually decreases with increase in temperature due to a decrease in yeast viability. But the yeast strain *S. bayanus* has been dominant in producing high percent of alcohol during mango wine production when compared to other yeast strains. These results correlated with the previous reports that yeast viability decreases as the temperature increases (Gao and Fleet 1988; Jones and Ingledew 1994). Temperature affected not only the fermentation kinetics but also the yeast metabolism, which determined the chemical composition and, in turn, the quality of the wine. As observed earlier, the final concentration of alcohol reduced as the temperature increased. It may be due to the increased concentration of byproducts from other metabolic pathways such as glycerol, acetic acid (Yannam et al. 2009, Naresh et al. 2014).

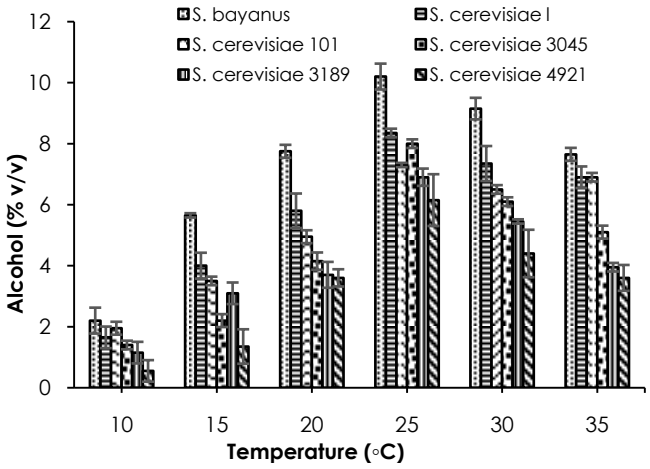

Figure 2: Effect of temperature on alcohol (% v/v) production in mango wine by six yeast strains.

Effect of sugar and alcohol concentration on yeast growth

During fermentation, sugars lead to the production of ethanol and carbon dioxide. Increasing the concentration of ethanol delays the growth of the yeast, which eventually stops the fermentation (Jimenez and Benitez 1987). It is desirable that the yeast strain used should be able to tolerate the highest sugar and ethanol concentration during fermentation. For beer the ethanol concentrations range between 3–9%, for grape wine 11–15% and for honey wine to 17% (Teramoto et al. 2005). The results obtained in alcohol tolerance of various yeast strains are presented in the following Table 1. The yeast viability was estimated after incubation of yeast cells in solutions with different ethanol concentration (from 6 to 20 % v/v). A cell suspension (1.97–2.1×10^8 cell/ml) was incubated at 20 °C under agitation (150 rpm) for 2 h in different ethanol concentrations. The resistance to 16% v/v alcohol concentration was estimated for the six yeast strains. There was a sharp decrease in cell viability with yeast. Among the six strains of wine yeast *S. bayanus*, *S. cerevisiae* (I), *S. cerevisiae* (101) and *S. cerevisiae* (3215) selected as they were able to grow in 10% (v/v) ethanol concentration and above. Only *S. bayanus* showed more tolerance to ethanol than the other species of the *S. cerevisiae* (Table 1).

It has been observed that all the strains of the *Saccharomyces* species were able to grow at all the sugar concentrations (Fig. 3) tested. Except for *S. bayanus* which recorded its highest growth rates in 20% (w/v), all other yeast strains had their maximum growth rates in the sugar concentrations of 15% (w/v). The growth rate remained the lowest in 25% (w/v) of sugar concentrations in all the yeast strains except *S. bayanus*. Comparatively, a higher viability was shown by the strain *S. bayanus* than the other strains. Only slight differences were observed in the growth rates (viability) with increasing sugar concentrations, the differences being most obvious between 10 and 25% sugar concentrations (Fig. 3).

The pattern of the effect of increased glucose concentration on *S. cerevisiae* was similar to that of *S. bayanus*. Increasing sugar concentrations for these strains prolonged their growth rate in the media containing 20 and 25% glucose, respectively. Also, reduced growth rates were equally observed in these media with an increase in sugar concentrations.

The ability of the five ethanol-tolerant yeast strains to withstand osmotic stress has been demonstrated by the fact that the yeast strains were able to grow in media containing relatively high degree of sugar concentrations. This observation is in agreement with the suggestion of Gray (1945) who stated that ethanol tolerant yeasts tend to be sugar-tolerant. Ekunsanmi and Odunfa (1990) asserted that the combination of sugar tolerance and alcohol tolerance is an advantage when a yeast is being considered for industrial use especially where ethanol is being produced. Jimenez and Benitez (1986) and Du Preez et al. (1987) pointed out that ethanol tolerance is

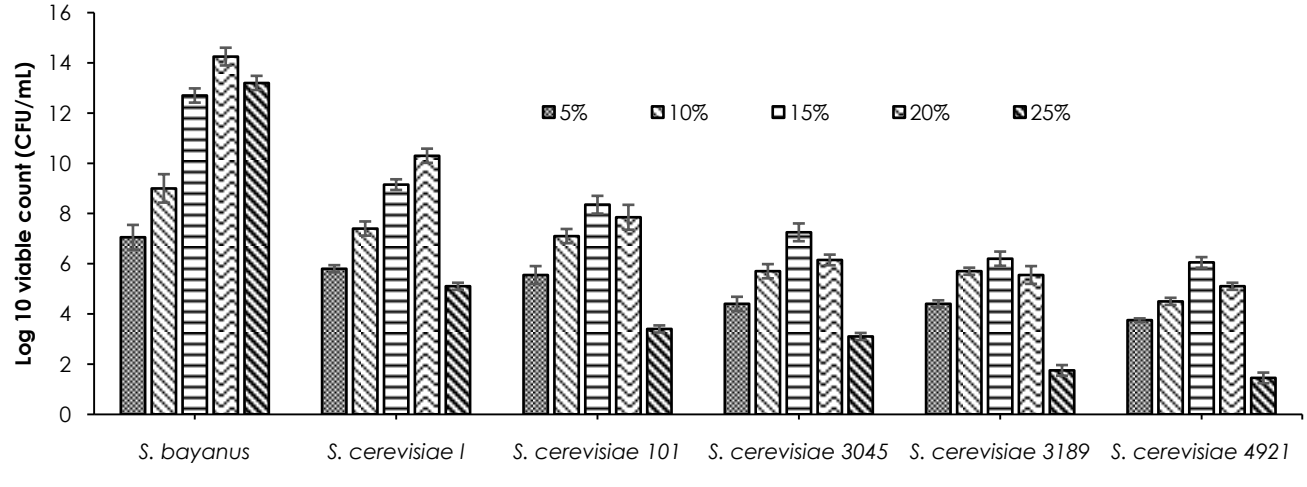

Figure 3: Effect of sugar concentration on the growth of different yeast strains

Table 1: Ethanol tolerance of yeast strains

Yeast strain	Ethanol tolerance (% v/v)							
	6	8	10	12	14	16	18	20
S. bayanus	+++	+++	+++	+++	++	+	-	-
S. cerevisiae (I)	+++	+++	++	+	-	-	-	-
S. cerevisiae (CFTRI 101)	+++	++	+	-	-	-	-	-
S. cerevisiae (NCIM 3215)	+++	++	+	-	-	-	-	-
S. cerevisiae (NCIM 3189)	+++	+	-	-	-	-	-	-
S. cerevisiae (NCIM 3045)	+++	+	-	-	-	-	-	-

Intensive growth (+++); Moderate growth (++); Low growth (+); No growth (-)

Table 2: Physico-chemical characteristics of mango wine produced by different yeast strains

Yeast strain	Ethanol (% v/v)	Total acidity (g/L)	Volatile acidity (g/L)	pH	Residual sugars (g/L)	Higher alcohols (mg/L)	Total esters (mg/L)
S. bayanus	10.5±0.5	5.5±0.05	1.5±0.01	3.7±0.1	3.8±0.06	286±12	28±3
S. cerevisiae (I)	6.9±0.2	4.3±0.12	1.48±0.03	3.9±0.1	4.2±0.01	224±9	26±9
S. cerevisiae (CFTRI 101)	6.5±0.4	5±0.09	0.94±0.01	3.7±0.2	3.7±0.02	321±23	31±6
S. cerevisiae (NCIM 3215)	5.8±0.1	6.2±0.05	1.20±0.02	4.0±0.1	4.5±0.07	196±14	16±2
S. cerevisiae (NCIM 3189)	5.3±0.3	7.7±0.14	1.60±0.05	4.0±0.3	4.9±0.03	240±18	23±1
S. cerevisiae (NCIM 3045)	4.8±0.2	8.4±0.05	1.80±0.01	3.6±0.2	5.3±0.02	161±8	11±5

particularly important since ethanol tolerance can hardly be avoided during fermentation although substrate inhibition can be avoided by the stepwise addition of substrate. In a medium where wine is the ultimate product, sugar tolerance by the wine yeast strains will allow the larger initial amount of sugar to be used.

Physicochemical characteristics of mango wine produced from different wine yeast strains

The six wine yeast strains were further screened for mango wine production to study the physicochemical characteristics of the wines produced from different yeast strains (Table 2). The high alcohol production was found in wine produced from the *S. bayanus* (10.5% v/v) and lowest alcohol (4.8%) in wine from *S. cerevisiae* (3045). The total acidity, volatile acidity, pH, residual sugars, higher alcohols and esters values were in comparable amounts in all the wines. The pH value of the wines ranging from 3.6-3.7 for all wines falls within the range of the recommended optimal pH level of 3.5-4.0 for wine fermentation (Amerine et al. 1980).

Rapp and Mandery (1987) found that total higher alcohols in wine were found to be in the range of 80-540 mg/l and concentrations upto 300 mg/l contributed to pleasant flavor, but above this concentration provoked unpleasant flavor and harsh taste. Thus, the wines made from all the strains have shown comparable amount higher alcohols, except in wine from *S. cerevisiae* 101 which has shown an increase in higher alcohol concentration (321 mg/l). The concentration of these components in wine is affected by many factors like variety of fruit, clarification and fermentation conditions. Other higher alcohols in high concentration, particularly isoamyl alcohol, contribute to unpleasant flavor (Kourkoutas et al. 2004). Kunkee and Vilas (1994) reported that the synthesis of acetic acid, isobutanol, and iso-amyl alcohol during fermentation depended primarily on yeast strain. Also, they have suggested that the yeast strain plays a decisive role in the formation of fusel oils or higher alcohols.

In addition, of higher alcohols, the production of esters also depend on various factors like fruit variety, yeast strain, and fermentation conditions. In the present study, the ester concentration is very much different from the wines produced with different yeast strains. Reddy and Reddy (2005) have also observed similar results. It clearly shows that the ester concentration is influenced by yeast variety and other environmental conditions. In the present study, the amount of ester production varied with all the six yeasts. The formation of volatile compounds during alcoholic fermentation depends not only on the particular species but also on the particular strain of the species. Selected strains of *S. cerevisiae* are now used in wine production in many countries and the results have been found to be excellent. The composition and quality of wine are closely related to the yeast strain used.In the present study, it has been found that *S. bayanus*is better than other strains for mango wine production.

Conclusions

The quality and quantity of physicochemical compounds found in mango wine depend upon the yeast strain used in this study. Therefore, it is important to know the potential differences in physicochemical compounds production by various yeast strains in order to select the best one to produce desirable wine. The results show that the yeast strain *S. bayanus* showed better performance than the other yeast strains with different pH, temperature and sugar concentrations. *S. bayanus* shows high ethanol tolerance than the other wine yeast strains. The average ethanol production by *S. bayanus* in wine was 10%. Together with the high glycerol and low volatile acid concentrations which imply the good quality attributes of wine produced by *S. bayanus*. Thus, the results obtained in this study will be beneficial for the effective production of high-qualitywine from mango fruits.

References

Alfenore S, Molina-Jouve C, Guillouet SE, Uribelarrea JL, Goma G, Benbadis L (2002) Improving ethanol production and viability of Saccharomyces cerevisiae by a vitamin feeding strategy during fed-batch process. Appl Microbiol Biotechnol 60:67–72.

Amerine MA, Berg HW, Kunkee RE, Ough CS, Singletons VL, Webb AD (1980) The technology of Winemaking, 4th Ed., AVI Publishing Co., Inc., Westport, CT.

Apetrei C, Apetrei IM, Nevares I, Del Alamo M, Parra V, Rodríguez-Méndez ML, De Saja JA. (2007) Using an e-tongue based on voltammetric electrodes to discriminate among red wines aged in oak barrels or aged using alternative methods: correlation between electro chemical signals and analytical parameters. Electrochim Acta 52:2588-2594.

Degree R (1993) Selection and commercial cultivation of wine yeast and bacteria, In: Fleet GH. (ed.): Wine Microbiology and Biotechnology. Harwood Academic Publishers, Chur: 421–448.

Dombek KM, Ingram LO (1987) Ethanol production during batch fermentation with *Saccharomyces cerevisiae*: Changes in glycolytic enzymes and internal pH. Appl Environ Microbiol 53:1286-1291.

Du Preez JC, Bosch MN, Prior BA (1987) Temperature profiles of growth and ethanol tolerance of the xylose fermenting yeasts Candida shematae and Pichia stipitis. Appl Microbiol Biotechnol 25:521-525.

Ekunsanmi TJ, Odunfa SA (1990) Ethanol tolerance, sugar tolerance and invertase activities of some yeasts strains isolated from steep water of fermenting cassava tubers. J Appl Bacteriol 69:672-675.

Gao G, Fleet GH (1988) The effects of temperature and pH on the ethanol tolerance of the wine yeasts, *Saccharomyces* cerevisiae, Candida stellata and Kloeckera apiculata. J Appl Bacteriol 65:405-409.

Gray WD (1945) The sugar tolerance of four strains of distillers' yeast. J Bacteriol 49:445-452.

Jimenez J, Benitez T (1986) Characterization of wine yeasts for ethanol production. Appl Microbiol Biotechnol 25:150-154.

Jimenez J, Benitez T (1987) Adaptation of yeast cell membranes to ethanol. Appl Environ Microbiol 53:1196–8.

Jones AM, Ingledew WM (1994) Fuel alcohol production: Optimization of temperature for efficient very-high-gravity fermentation. Appl Environ Microbiol 60:1048-1051.

Kourkoutas Y, Bekatorou A, Banatb IM, Marchant R, Koutinas AA (2004) Immobilization technologies and support materials suitable foralcohol beverages production: a review. Food Microbiol 2:377-397.

Kunkee RE, Vilas M (1994) Towards an understanding of the relationship between yeast strain and flavour production during vinifications: flavour effects in vinifications of non distinct variety of grapes by several strains of wine yeast. Vatic Enol Sci 49:46-50.

Miller GL (1959) Use of dinitrosalicylic acid for determination of reducing sugar. Anal Chem 31:426-428.

Naresh Kondapalli, Varakumar Sadineni, Prasad Shekhar Variyar, Arun Sharma, Vijaya Sarathi Reddy Obulam (2014) Impact of γ-irradiation on antioxidant capacity of mango (Mangifera indica L.) wine from 8 Indian cultivars and protection of mangowine against DNA damage caused by irradiation. Process Biochem 49:1819-1830.

Pretorius IS (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast16:1-55.

Pretorius IS, Toit M, Rensburg P (2003)Designer Yeasts for the Fermentation Industry of the 21 st. Century. Food Technol Biotechnol 41:3–10.

Raineri S, Pretorius IS (2000) Selection and improvement of wine yeast. Ann Microbiol50:15– 30.

Rapp A, Mandery H (1987) New progress in wine and wine research. Experientia 45:873-884.

Reddy LVA, Reddy OVS (2005) Production and characterization of wine from mango fruit (Mangifera indica L.). World J Microbiol Biotechnol 21:1345–1350.

Ribéreau-Gayon P, Dubourdieu D, Doneche B, Lonvaud A (2000) Handbook of Enology. John Wiley & Sons Ltd, London.

Romano P, Monteleone E, Araggio M, Marchese R, Caporale G. Carlucci A (1998) A methodological approach to the selection of Saccharomyces cerevisiae strains. Food Technol Biotechnol36:69–74.

Sudheer Kumar Y, Varakumar S, Reddy OVS (2012) Evaluation of antioxidant and sensory properties of mango (Mangifera indica l.) wine. J Food 10:12-20.

Teramoto Y, Sato R. Ueda S (2005) Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. Afr J Biotechnol 4:660–2.

Wieland OH(1988) Glycerol. In Methods of Enzymatic Analysis(Bergmeyer, H. U., ed.), 3rd(4), 504-510, VCH Publishers (UK) Ltd., Cambridge, UK.

Yannam Kumar, Reddy Shetty Prakasam, Obulum Vijaya Sarathi Reddy (2009) Optimization of fermentation conditions for mango (Mangifera indica L.) wine production by employing response surface methodology. Int J Food Sci Technol 44:2320-2327.