Original Research Article

Effect of yeast concentration on quality parameters of ber (*Ziziphus mauritiana*) fruit (cv. *Umran*) wine during ageing

Abstract :

The present investigation was carried on for production of ber wine using yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*)forfermentation. The quality parameters of ber wine were predicted by Design-Expert 7.7.0 software for optimizing the process parameters. The results obtained at T_4 treatment (5 % inoculum) were encouraging with TSS (7.61 Brix), pH (4.06), residual reducing sugars (5.22 %) and alcohol (10.92 %) on 0^{th} day of ageing with desirability factor of 0.95 followed by the results obtained on 90^{th} day of ageing for T_4 treatment (5 % inoculum) were TSS (6.27 °Brix), pH (4.22), residual reducing sugars (4.71 %) and alcohol content (12.07 %) with desirability factor of 0.93.

Key words: Ber, Fermentation, Wine, Quality Parameters and Ageing

Introduction

Wine is one of the oldest alcoholic beverages dating back to the Egyptians, almost 5000 years ago (Pretorius, 2000). Until the early years of the seventeenth century, wine was considered to be the only wholesome readily storable beverage accounting for rapid global increase of wine fermentation technology (Pretorius, 2000). Wine plays a major role in the economy of many nations. It is generally accepted that, moderate wine drinking is socially beneficial and can be effective in the management of stress and reduction of coronary heart disease.

Wine is one of the functional fermented foods and has many health benefits. These include anti-ageing effects in red grape skin, improvement of lung function from antioxidants in white wine, reduction in coronary heart disease, development of healthier blood vessels in elderly people, reduction in ulcer-causing bacteria, destruction of cancer cells by protein present in red grape skin, prevention of stroke by keeping the arteries clean by polyphenols in red grape skin, decreasing ovarian cancer risk in women and making the bones stronger.

Many wines are made from fruits having medicinal value and such wines have many additional benefits (Soni *et al.*, 2009).

Ber is a tropical and subtropical fruit native to the Northern hemisphere. It belongs to the Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Rosidae, Order: Rhamnales, Family: Rhamnaceae, Genus: *Ziziphus mill*, Species: Z*iziphus mauritiana Lam*. (Pareek *et al.*, 2009). Ber is previously recognized as poor man's fruit, also designated as "King of arid fruits" owing to the fact that it can be grown in unproductive, waste, marginal or inferior soil with pH as high as 9.0 in arid and semi-arid regions which are characterized by extreme variations of diurnal annual temperatures and high evaporations coupled with sparse and highly variable precipitations.

In the present investigation the attempts were made to prepare the wine from ber fruit with the objectives to check the optimized of quality parameters during ageing of ber wine.

Materials and Methods

Procurement of raw materials

Fresh ber fruits (*Ziziphus mauritiana*) of variety Umran were procured from the APMC market, Bagalkot for conducting the experiment.The fresh harvested fruits were washed with potable water to remove the dust, dirt and foreign materials. The seeds were removed by using hand operated fruit de-seeder and the fruit was crushed by using power operated fruit pulper to obtain juice. The process flow chart adopted for production of wine from ber fruit is shown in Fig. 1

Collection of ber fruits

Cleaning and washing

Deseeding and crushing

Juice extraction

Enzyme pre-treatment (with 1 % pectinase)

Selection of best sample for fermentation process

(Improved reducing sugars content)

□

Adjustment of TSS at 24 °Brix (Pandhre*et al*., 2010)

□

pH adjustment (4.0 pH) (Younis *et al*., 2014)

□

Addition of KMS (@ 200ppm to inhibit the growth of wild yeast and spoilage organisms)

□

Yeast inoculation

(0 %, 2 %, 3 %, 4 % and 5 % inoculum)

□

Aerobic fermentation for 2 days

□

Anaerobic fermentation for 14 days

□

Addition of bentonite clay (400 mg/l)

□

Siphoning / straining (2-3 times) to get clear wine

□

Filling in sterile bottles

□

Ageing of wine at cold storage condition (5 ± 2 °C)

(Observed the quality parameters at 15 days interval from 0, 15, 30, 45, 60, 75 and 90 days)

Fig. 1: Process flow chart for production of wine from ber fruit

Ageing of wine

The ber wine obtained from different treatments was bottled, sealed and stored in a cold condition by maintaining a average temperature of 5 ± 2 ºC. The stored wine was used to study the quality parameters at every 15 days of interval and the organoleptic quality of wine was tested after three months of duration (Lokesh *et al.*, 2014).

Analysis of quality parameters of prepared wine

The wine was analyzed for different quality parameters *viz.,* TSS, pH, colour, titratable acidity, ascorbic acid, residual reducing sugars and alcohol content by following standard procedures.

Total soluble solids

The total soluble solids (TSS) of ber wine were determined as per the method described by Joshi *et al*., 2012, using digital handheld refractometer. The instrument was calibrated by cleaning and adjusting initial value zero at 20 °C using distilled water. Appropriate quantities of wine sample were placed on the prism of the refractometer with the help of a glass rod and press the start button to get the readings. For each sample, the instrument was calibrated by using distilled water. The reading appeared on the screen was directly recorded as total soluble solids (°Brix).

pH

The pH of prepared ber wine was measured by using digital pH meter. Accurately weighed 5 ml wine sample was placed in a beaker and then an electrode of pH meter was dipped in the wine sample. The enter key was pressed to show the pH and temperature values of sample simultaneously. For the subsequent samples, the electrode was removed and washed properly with distilled water. The above procedure was repeated for all the four samples (Fustier *et al*., 2011).

Colour

Colour is one of the most important quality acceptances parameters for any food product. Hunter's lab colourimeter was used for the measurement of colour values of prepared wine. The colour was measured by using CIELAB scale at 10° observer at D_{65}illuminant. It works on the principle of focusing the light and measuring the energy reflected from the sample across the entire visible spectrum. It provides reading in terms of L^*, a^* and b^* values. Where, luminance (L^*) forms the vertical axis, which indicates whiteness (+) to darkness (-). In the same way, a^* indicates redness (+) to greenness (-) and b^*indicates yellowness (+) to blueness (-) Liu *et al.,* 2015.

Titratable acidity

Ten ml of wine sample was taken in a volumetric flask and the volume was made up to 100 ml. From this, 10 ml of aliquot was taken in a 100 ml conical flask and titrated against 0.1 N NaOH using one or two drops of phenolphthalein indicator. Appearance of light pink colour was noted at the end point. Total titratable acidity was expressed as per cent citric acid (Srivastava and Kumar, 1993).

$$\text{Titratable acidity (\%)} = \frac{\text{Titrablevalue} \cdot \text{Normality of NaOH} \cdot \text{Vol. made up} \cdot \text{equivalant weight of acid}}{\text{Vol. of sample for estimation} \cdot \text{Weight or volume of sample taken x 1000}}$$

Ascorbic acid

The wine samples were analyzed for the ascorbic acid content, using 2, 6-Dichlorophenol indophenol dye titrimetrically as per method suggested by Sadasivam and Manickam (1992). Two grams of sample weighed in weighing balance was blended with 10 ml of 4 per cent oxalic acid and filtered through muslin cloth. An aliquot of extract (2 ml) of the sample was titrated against 2, 6-Dichlorophenol indophenol dye till the pink end point persisted for at least 15 seconds (TV_2). Similar procedure was followed for acid mixture to get blank titre value and against standard solution made in 4 per cent oxalic acid to get standard titre value (TV_1). The result expressed in terms of mg/100 g.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Ascorbic acid in standard x } TV_2 \text{ x Total sample volume}}{\text{Volume taken x } TV_1 \text{ x Weight of the sample}} \times 100$$

Residual reducing sugars

The method as suggested by Nelson Somogyi (Sadasivam and Manickam 1992) was used for estimation of reducing sugars. One ml of each treated sample was taken in a test tube to which one ml of alkaline copper reagent was added and heated for 20 minutes in boiling water bath and then cooled. To this, one ml of Arsenomolybdate reagent was added and volume was made up to 10 ml. The absorbance was read at 510 nm using UV- visible Spectrophotometer. Standard curve was obtained from glucose standards (10-100 µg) and from the standard curve milligram of reducing sugars was determined and the final amount of reducing sugars in the sample was calculated.

Alcohol content

An Ebulliometer instrument was used for determination of the alcohol content of water-alcohol solutions by determining the difference in boiling points between pure water and the solution. Based on the comparison, the percentage alcohol (v/v) was determined (Buescher *et al.,* 2011).

Optimization of quality parameters during ageing of ber wine

The numerical optimization techniques of the Design-Expert software were used for the simultaneous optimisation of the quadrupleresponses. The desired goals for each variable and response were chosen. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. All of the independent variables were kept within range, while the responses were either maximised or minimised. Numerical optimization was applied for TSS, pH, residual reducing sugars and alcohol on the basis of quality parameters of wine.

The quadratic response surface analysis was based on multiple linear regressions taking into account linear, quadratic and interaction effects according to the equation below:

$$Y = b_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^{\,2}$$

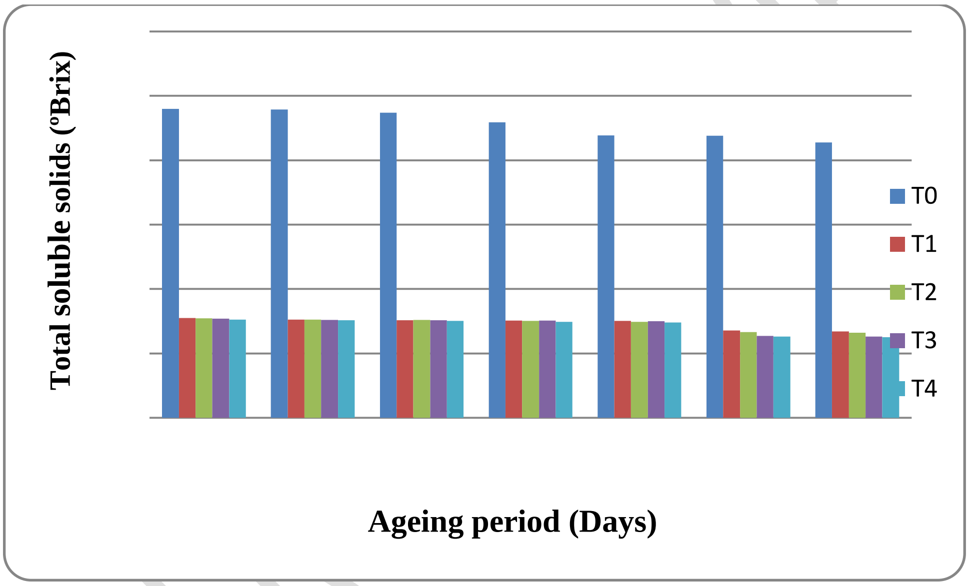
Where Y is the response value predicted by the model; b_0 is offset value, a_i, a_{ij} and a_{ii} are main (linear), interaction and quadratic coefficients, respectively. The adequacy of the models was determined using model analysis; lack-of fit test and coefficient of determination (R^2) analysis. For model to be suited, R^2 should be at least 0.80 for a good fitness of a response model (Panesar *et al*., 2009).

Results and Discussion

Analysis of quality parameters of ber wine

Total soluble solids (TSS)

The effect of yeast concentration on TSS (°Brix) of ber wine during ageing is shown in Fig. 2. It is observed that with the increase in yeast concentrations and number of days after treatment, the TSS in wine decreased. The highest TSS was observed in treatment T_0 at 0 per cent yeast with 23.99 brix on 0^{th} day of ageing and the lowest was observed in treatment T_4. The maximum total soluble solid after 90^{th} day of ageing was recorded as 21.38 ºBrix in control, where as the minimum was recorded as 6.27 ºBrix in treatment T_4 at 5 per cent yeast concentration. The reduction in the TSS during ageing of wines is expected to be due to the slower yeast activity that may still prevail during ageing which converts sugars into alcohol Kinnow wine showed a decrease in TSS from 24.0 to 8.0 °Brix (Pratima *et al.*, 2006). Jairath *et al.* (2012) reported TSS of amla wine varied from 16.0 to 13.0 ºBrix.



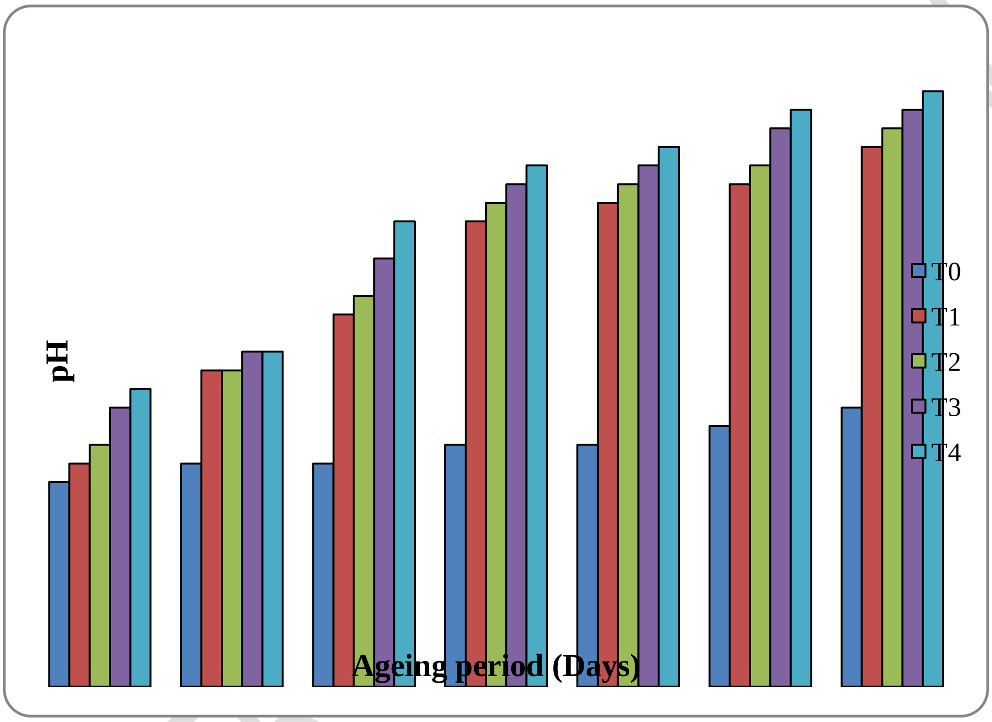
T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 2: Effect of yeast concentration on TSS (°Brix) of ber wine during ageing

Acidity (pH)

It is evident from the Fig. 3. that, for all the treatments there was an increase in pH value with the increased ageing and the concentrations of yeast. The results showed that, the pH of ber wine ranged from 4.01 to 4.06 at the end of fermentation (0^{th} day of ageing). The maximum pH after 90^{th} day of ageing was 4.22 in treatment T_4 at 5 per cent yeast concentration, whereas the minimum of 4.05 was observed in control.

The increase in pH might be due to enhanced synthesis of esters from ethyl alcohol and volatile acids. The change in the pH was not correlated with the change in total acidity because of the buffering capacity of the wine and the relative amount of various acids influencing the acidity (Shankar *et al.,* 2004). The pH of the wine during ageing showed an increasing trend. Similar observations of increase in pH after fermentation and ageing were reported by Jairath *et al.* (2012) in guava wine (3.1) and Soni *et al*. (2009) in Amla wine (3.52).

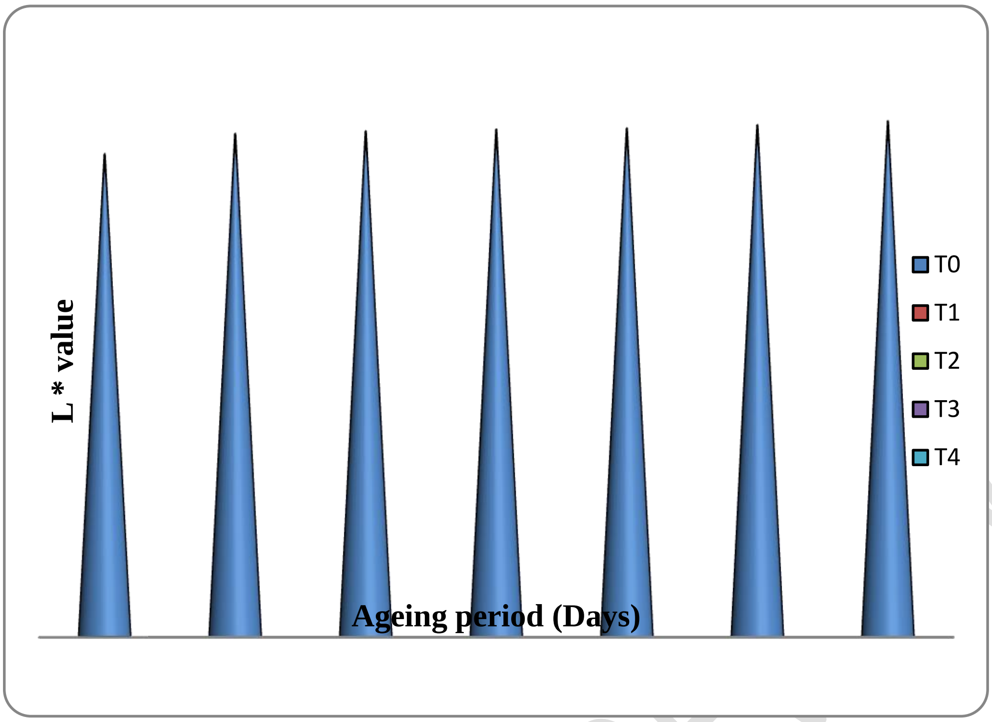


T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 3: Effect of yeast concentration on pH of ber wine during ageing

***L^** value**

The effect of yeast concentration and ageing on L^* value of ber wine is presented in Fig. 4. Among the treatments, T_1 (2 % yeast concentration) recorded the highest L^* value 3.22 on 0^{th} day of ageing. It was observed that, for all the treatments, there was an increase in L^* value with the ageing process. The highest L^* value after 90^{th} day of ageing was recorded as 5.78 for treatment T_1 and the lowest was recorded as 4.25 in T_4. It was observed that, the L* value increased with the increase in ageing process,which means it lost colour intensity. Similar results for L* value duringageing process were also observed by Liu *et al*. (2015) on bilberry syrup wines.

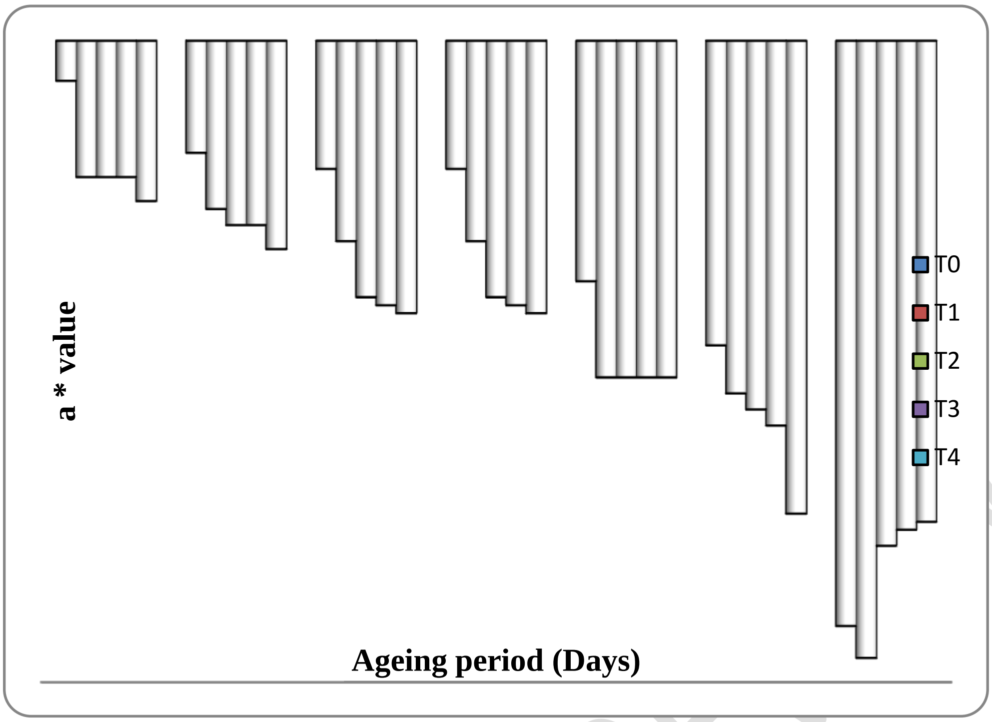


T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 4: Effect of yeast concentration on colour *L value of ber wine during ageing**

***a** value**

The data on the effect of yeast concentration and ageing on a^* value of ber wine during ageing is presented in Fig. 5. It was observed that, the a^* values of ber wine varied from -0.20 to -0.17 on 0^{th} day of ageing. The highest a^* value (-0.60) was recorded in treatment T_4 at 5 per cent yeast concentration and the lowest a^* value recorded as -0.77 in treatment T_1 after 90^{th} days of ageing. From the figure, it was observed that, the a^* value decreased with the increase in ageing process which might be due to the rise of pH value, the flavylium cations lose protons forming the quinoidal base, at the same time, the flavylium cation is hydrolyzed into the hemiketal or carbinol pseudo-base (colourless), and the hemiketal or carbinol pseudo-base slowly ring opens into a chalcone (He *et al.*, 2012). Similar results of increased a^* value with the increase in ageing period were reported by Liu *et al*. (2015) on bilberry syrup wines.

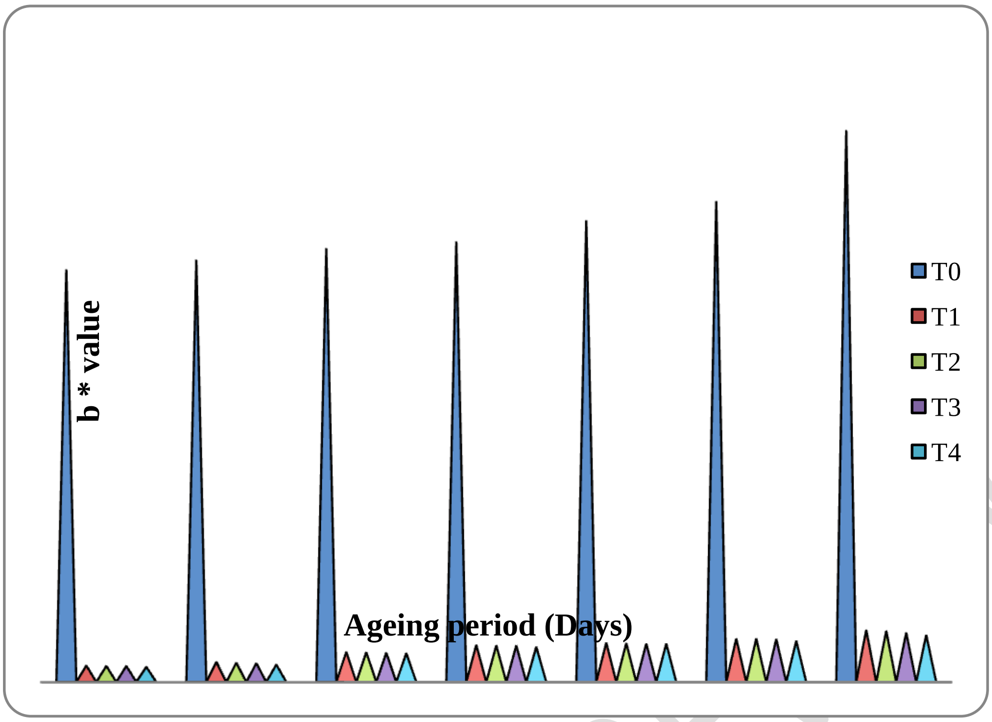


T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 5: Effect of yeast concentration on colour *a value of ber wine during ageing**

***b** value**

The effect of different inoculum levels and ageing on b^* value of ber wine is presented in Fig. 6. It was observed that, the b^* value of ber wine was highest (9.01) in control as compared to other treatments. The maximum b^* value after 90th day of ageing was recorded as 1.14 for treatment T_1 and the lowest was recorded as 1.03 in T_4. It was observed that, for all the treatments there was an increase in the *b** value with increase in ageing period. b* value with ageing time were associated with the formation of yellow-orange pigments like pyranoanthocyanins and also due to the oxidation (Rentzsch*et al.*, 2010). Similar results of *b** valuewere also reported by Liu *et al*. (2015) onbilberry syrup wines.



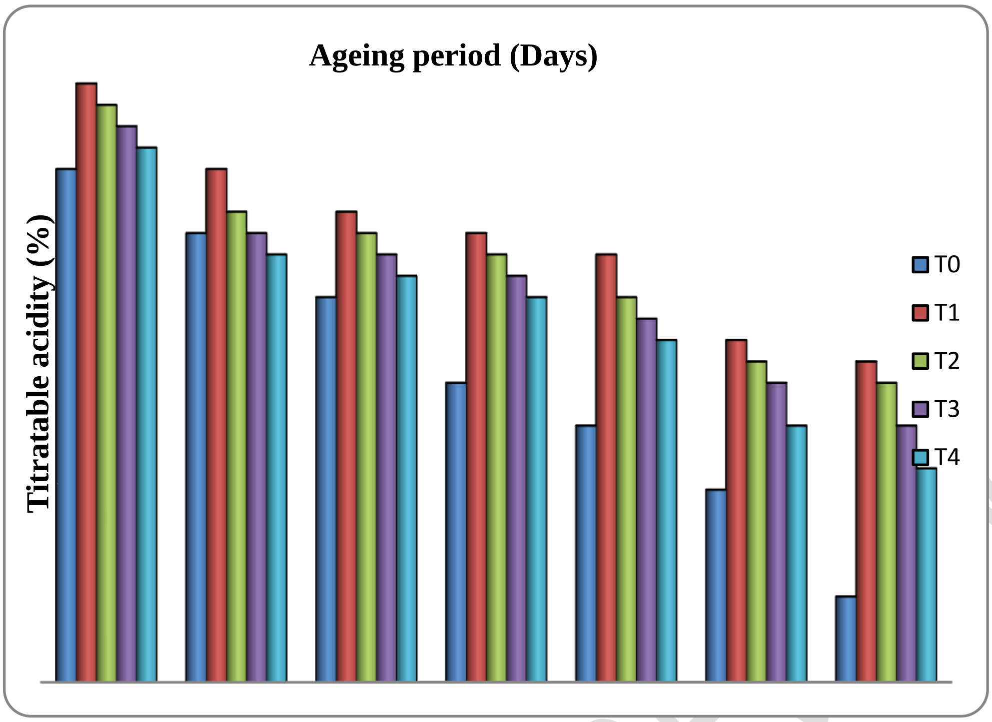
T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 6: Effect of yeast concentration on colour *b value of ber wine during ageing**

Titratable acidity

The effect of yeast concentration on titratable acidity during ageing is presented in Fig. 7. The titratable acidity of ber wine ranged from 1.0 to 1.12 per cent on 0^{th} day of ageing, whereas in control it was recorded as 0.96 per cent. Wine that had undergone 90^{th} of ageing in cold condition (5 ± 2 ºC) showed minimum titratable acidity 0.40 per cent in treatment $T_{4,}$ as compared to control (0.16 %).

The changes in pH were correlated with the changes in titratable acidity because of the buffering capacity of the wines and the relative amounts of various acids influencing the acidity (Amerine *et al.*, 1972). The decrease in the acidity during ageing might be due to combination of acids with alcohol to form esters which adds aroma to the wine during ageing (Shankar *et al.,* 2004). It was observed that, there was a decrease in titratable acidity during ageing. The minimum titratable acidity 0.40 per cent was a recorded in treatment $T_{4.}$ Similar result was recorded by Pandhre*et al.* (2010) in banana wine (0.44 %). The variations in the titratable acidity due to similar treatment were accounted in strawberry wine (Somesh *et al.,* 2009).



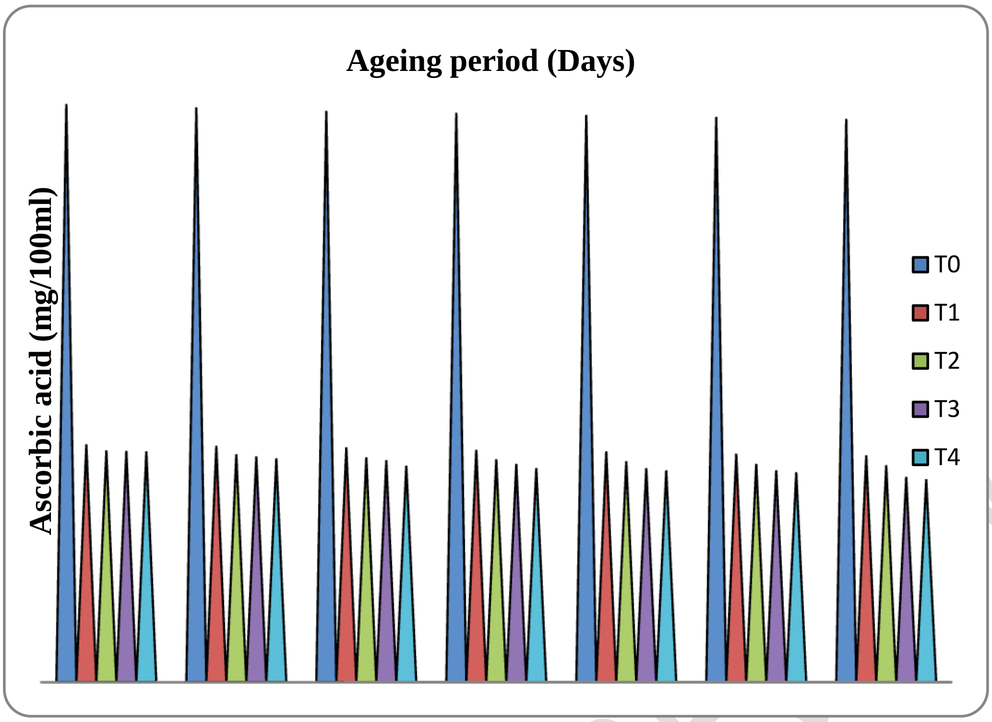
T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 7: Effect of yeast concentration on titratable acidity of ber wine during ageing

Ascorbic acid

The effect of yeast concentration on ascorbic acid content of ber wine during ageing was determined and presented in Fig. 8. The results showed that, the ascorbic acid content of ber wine ranged from 25.18 to 25.94 mg/100 ml on 0^{th} day of ageing. The maximum ascorbic acid (24.75 mg/100 ml) after 90^{th} day of ageing was recorded in treatment T_1, whereas the minimum (22.15 mg/100 ml) was recorded in treatment $T_{4.}$ Significant declining trend in ascorbic acid content was observed during ageing.

Decrease in ascorbic acid content of wine during ageing observed in present study (Fig. 8) might be due to the native ascorbic acid content of ber fruit decreases trend with increase in ripeness of fruit (Pawar *et al.*, 2011). Results of the present study are supported by findings of Jairath *et al*. (2012) in amla wine (90.0 mg/100 ml) and Kocher and Pooja (2011) in guava wine (63.0 mg/100 ml).



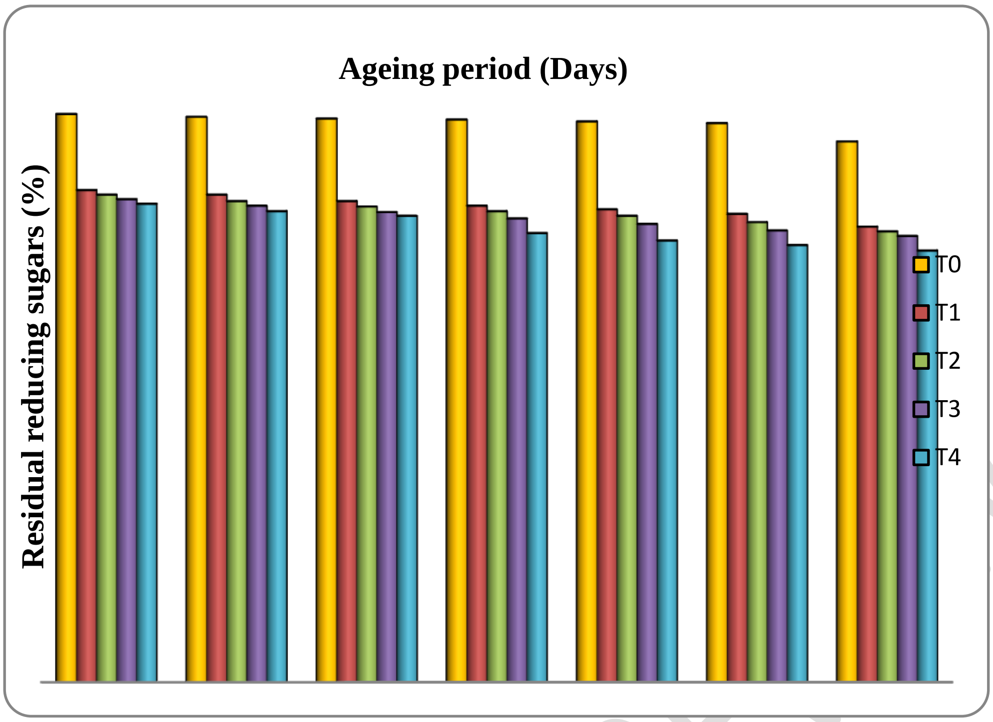
T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 8: Effect of yeast concentration on ascorbic acid of ber wine during ageing

Residual reducing sugars

Residual reducing sugars of ber wine subjected to different yeast concentration during ageing is depicted in Fig. 9. It was found that the residual reducing sugars of ber wine was found to vary significantly among the treatments. The results showed that, the residual reducing sugarscontent of ber wine ranged from 5.22 to 5.37 per cent on 0^{th} day of ageing. The maximum residual reducing sugars (4.97 %) after 90^{th} day of ageing was recorded in treatment T_1, where as the minimum (4.71 %) was recorded in treatment $T_{4.}$

The low content of residual reducing sugars indicates that almost all the reducing sugars were consumed during fermentation (Singh and Kaur, 2009). The indentical observations were reported by Lokesh *et al.* (2014) in jamun wine (4.72 %) and Jairath *et al.* (2012) in amla wine (5.75 %). The residual reducing sugars in jamun wine were also decreased with the increase of pH and yeast concentrations as reported by Sonar *et al.* (2004).



T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 9: Effect of yeast concentration on residual reducing sugars of ber wine during ageing

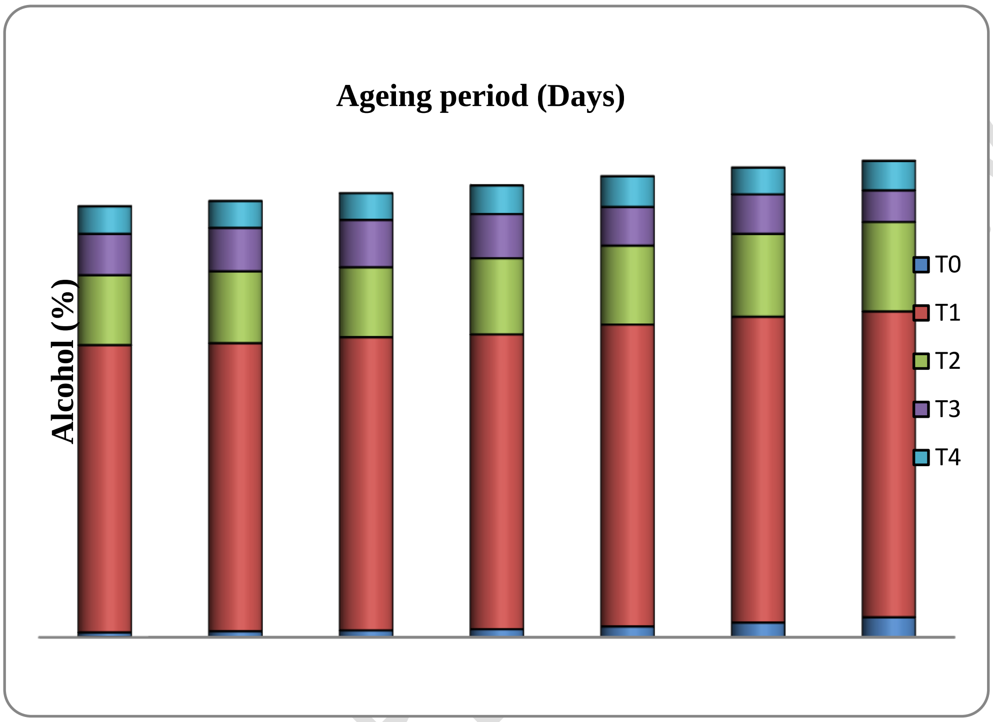
Alcohol content

The results revealed that, there was a significant difference in alcohol content in all the treatments (Fig. 10). It was observed that, there was an increase in alcohol content all the treatments during ageing. The results are in confirmative with the present findings by Sonar *et al.* (2004) that, the alcohol content was increased with the increase in yeast concentration and pH of must in jamun wine.

The results showed that the alcohol content of ber wine ranged from 7.40 to 10.92 per cent on 0^{th} day of ageing. The maximum alcohol per cent on 90^{th} day of ageing was recorded as 12.07 per cent in treatment T_4. The results are in agreement with the findings of Marimuthu and Thirumaron (2002) for fruits like ber and jamun. They observed maximum alcohol content in ber wine was 12 per cent.

The reason for increase in alcohol content in wine might also be due to utilization of sugars present in the yeast and converting them in to carbon dioxide and ethyl alcohol in anaerobic condition as reported by Carl (1979). During ageing, the alcohol level was increased slightly in all the treatments. This increase might be due to very slow fermentation that might have occurred during ageing. Similar trend of increase in alcohol content during ageing was reported by Tusekwa*et al.* (2000) in Tanzanian wine.

The increase in alcohol content (per cent) during ageing were also recorded by Lokesh *et al.* (2014) in jamun wine (7.92 %) and Pratima *et al.* (2006) in kinnow wine (12.2 %).



T_0 = 0 % yeast, T_1 = 2 % yeast, T_2 = 3 % yeast, T_3 = 4 % yeast, T_4 = 5 % yeast

Fig. 10: Effect of yeast concentration on alcohol content of ber wine during ageing

Optimization of quality parameters during ageing of ber wine

The responses as predicted by Design-Expert 7.7.0 software for the optimum quality parameters of wine with desirability factor after fermentation were analysed (Fig. 11). The optimized conditions were obtained for T_4 treatment with 7.61 °Brix, 4.06 pH, 5.22 per cent residual reducing sugars and 10.92 per cent alcohol on 0^{th} day of ageing. Whereas optimized conditions obtained on 90^{th} day of ageing for T_4 treatment were 6.27 °Brix, 4.22 pH, 4.71 per cent residual reducing sugars and 12.07 per cent alcohol with desirability factor 0.93 is shown in Fig. 12.

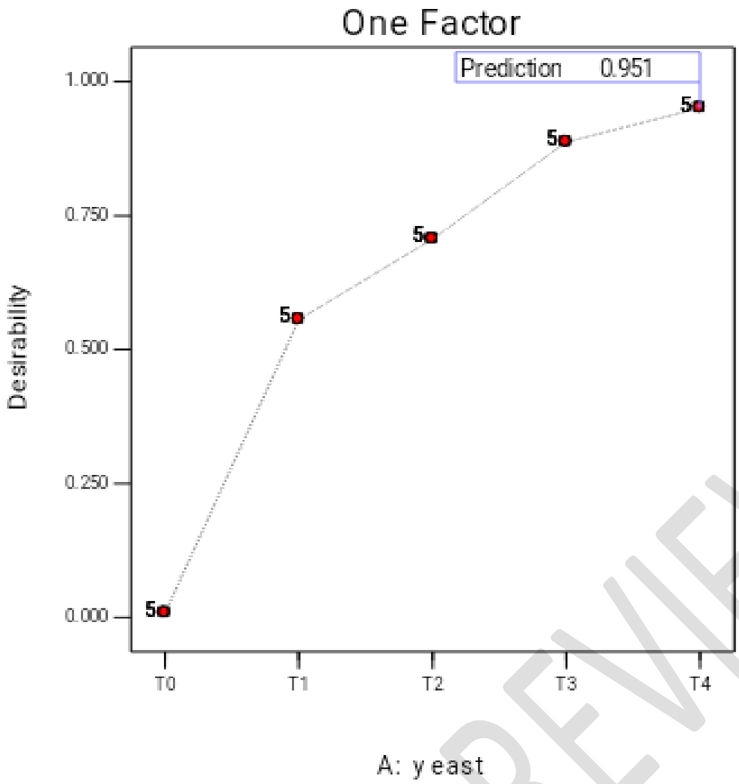


Fig. 11: Desirability of Optimization of yeast concentration on ber wine quality after fermentation

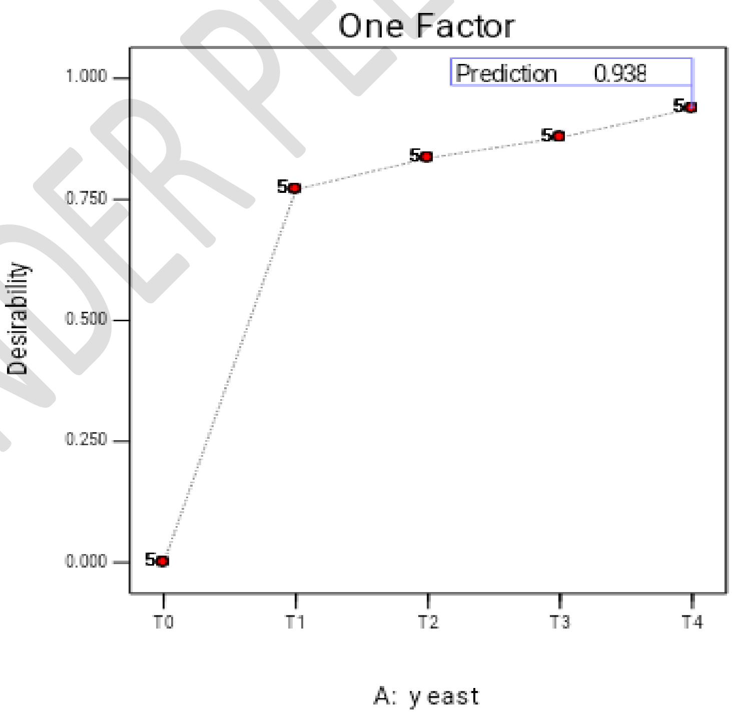


Fig. 12: Desirability of Optimization of yeast concentration on ber wine quality at 90th day of ageing

Conclusion

The quality parameters *viz*., TSS, pH, colour, titratable acidity, ascorbic acid, residual reducing sugars and alcohol of ber wine during ageing were studied.The optimization of quality parameters of ber wine as predicted by Design-Expert 7.7.0 software for optimum process conditions showed desirability of 0.95. The optimized condition was obtained at T_4 treatment with TSS (7.61 Brix), pH (4.06), residual reducing sugars (5.22 %) and alcohol (10.92 %) on 0^{th} day of ageing. Whereas optimized condition obtained on 90^{th} day of ageing were for T_4 treatment with TSS (6.27 °Brix), pH (4.22), residual reducing sugars (4.71 %) and alcohol (12.07 %) with desirability of 0.93.

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