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Production of wine from Mahua (*Madhuca indica* L.) flower extract and Pomegranate (*Punica granatum* L.) fruit juice

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Abstract

Wine is an alcoholic beverage typically made of fermented fruit juice. The fermentation process of fruit juice into wine is a complex biochemical reaction involving microorganisms that requires study and practice. Two strains of *S. cerevisiae* (NCIM-3206) and *S. cerevisiae* (NCIM-3215) were used for production of wine by fermentation of mahua flower extract and pomegranate fruit juice. Fermentation parameters to produce wine from 2 proportions namely A (10:90) and B (20:80) were optimized i.e. temperature (25°C, 30°C and 35°C), pH (3.5, 4.5 and 5.5), inoculums concentration (3%, 6% and 9%) and incubation period (24 hrs, 48 hrs and 72 hrs). After 7 days of fermentation it was observed that the strain *S. cerevisiae* NCIM 3215 showed better results than the yeast strain *S. cerevisiae* NCIM 3206 in case of overall physicochemical characteristics at the pH 4.5, temperature 30°C, inoculums concentration 6% and incubation period 24hr old starter culture for production of wine individually. Sensory evaluation revealed that wine produced by using *S. cerevisiae* (NCIM 3215) and *S. cerevisiae* (NCIM-3206) both were acceptable with respect to sensory and biochemical characteristics. Sensory evaluation rated the wine produced with all optimized parameters together by using *S. cerevisiae* (NCIM-3215) of B (20:80) proportion more acceptable.

Keywords: mahua, *S. cerevisiae*, NCIM 3215, NCIM 3206, proportions and fermentation

1. Introduction

1.1 Mahua

Mahua (*Madhuca indica*) is a large, deciduous tree native to the northern and central parts of India. Tribal populations across the country value the flowers of this tree as a source of food. The mahua flowers are one of the most underutilized nutrient sources due to their extremely rare consumption among the Indian populace. The flowers are nutritionally balanced as a rich source of carbohydrates, proteins, dietary fibers in addition to minerals like iron and calcium. The most interesting attribute of the mahua flowers is their long shelf life after drying. *Mahua* has many properties such as analgesic activity, antibacterial activity, hepatoprotective activity and antioxidant activity (Prasad *et al.*, 2015) [9].

1.2 Pomegranate

Pomegranate (*Punica granatum*) is a favorite table fruit in tropical and subtropical regions of the world which belongs to the family *Lythraceae*. India is the leading pomegranate producer and produces finest edible quality pomegranates. Pomegranate is being cultivated on an area of 1.31 lakh ha/hectares in India with production of 13.46 lakh tones (Sonawane, M. S., 2017) [12]. Pomegranate is useful for manufacturing wines rich in bioactive compounds (Mena *et al.*, 2012) [7]. It is also important to highlight the healthy effects described for pomegranate wines, probably linked to its phenolic composition (Schubert *et al.*, 1999) [11]. Pomegranate wine was produced in accordance to fermentation started after adding yeast and temperature was kept at 22 °C throughout the fermentation process (9 days) with a latex glove served as pressure CO₂ release valve. Once fermentation finished, the wines were clarified and racked for one day at 4 °C. Wines were left to stabilize for 10 days in darkness at 20 °C.

2. Materials and Methods

2.1 Experimental site

The present was carried out at the Department of Post-harvest and Food Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur

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2.2 Experimental material

2.2.1 Collection of Mahua flowers, pomegranate fruits and yeast strains

The experimental material included in present study consists of Mahua flower and Pomegranate fruits viz., Mahua flowers were collected from the tribal area of Kinwat, Nanded district of Maharashtra state. Yeast strains *S. cerevisiae* NCIM 3206 and NCIM 3215 were collected from National Chemical Laboratory, Pune in slant forms. The cultures are kept in refrigerator (4°C) until further use.

2.3. Methodology

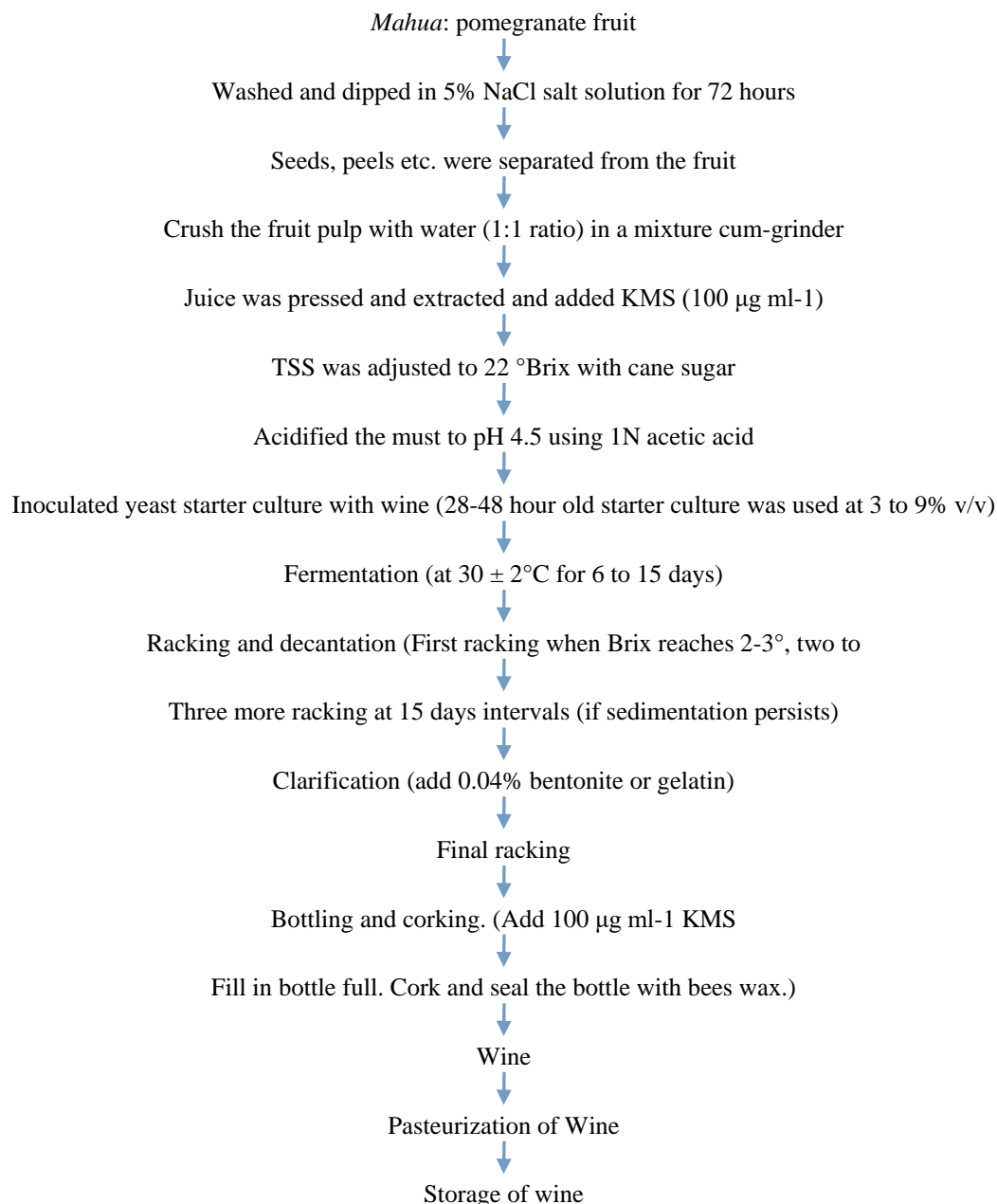
2.3.1 Multiplication and maintenance of yeast strains

The cultures were kept in refrigerator until used. The yeast inoculums were subcultured in YEPD broth and/or agar.

2.3.2 Preparation of mixed of mahua flower extract and pomegranate juice

The mahua flower extract and pomegranate fruit juice was extracted as given in following flow chart.

2.3.2.1 Flow chart for wine production



2.3.2.2 Blending proportion of mahua flower extract and pomegranate juice

The blending proportions were mixed in a conical flask in 2 proportions namely A (10:90) and B (20:80) respectively. After making the blends, the T.S.S was adjusted to 22°Brix. The acidity was adjusted by addition of citric acid. Potassium metabisulphate (50ppm) was added to the mixture to sterilize the juice. Then mixed sample (1L) was pasteurized at 82°-85°C for 20 minutes and stabilized by keeping at room temperature for 24 hrs.

2.3.2.3 Starter culture preparation

50 ml mixture of *mahua*: pomegranate juice was extracted and placed in the conical flask (250 ml) and pasteurized. Pasteurized juice sample was inoculated with two different species of pure culture of *S. cerevisiae* strains of NCIM 3206 and NCIM 3215 under aseptic conditions by using the laminar air flow system. The flasks were incubated at 28 ± 2 °C for different (24, 48 and 72 h) hours with constant agitation at 160 rpm. Then different (24, 48 and 72 h) hours old starter cultures were used for inoculation of various blends of

mahua: pomegranate extract in the ratio of 1:20 (starter culture: juice sample) for the preparation of wine.

2.3.2.4 Fermentation of the mixed extract of *Mahua*: pomegranate juice

New Brunswick Scientific BioFlo 110 fermenter was used.

2.3.2.5 Optimization of fermentation process for *mahua*: pomegranate juice

Various blended extracts of *mahua*: pomegranate were used for production of the wine by fermentation process by considering the following parameters.

For optimization of fermentation conditions, following treatment combinations were used- temperature of 25°C, 30°C and 30°C; pH of 4.5, 5.5 and 6.5; inoculum concentration of 3%, 6% and 9% along with differential incubation period 24 hrs, 48hrs and 72 hrs. The yeast extract was added as a nitrogenous source at the rate of 0.1 % (w/v) into the mixed juice. The pectin enzyme was added @ 0.5%. Inoculum concentration of 3%, 6% and 9% of yeast strains *S. cerevisiae* (NCIM 3206 and NCIM 3215) were used for differential incubation period of 24 hrs, 48 hrs and 72 hrs in the optimization process. These samples were transferred in a BOD incubator for fermentation and maintained at temperature 25 °C, 30 °C and 35 °C. Fermentation was carried out for 5 to 10 days till the T.S.S. reading got stable, then the product was collected into the sterilized cap bottle for further process.

2.3.2.6 Racking, clarification, centrifugation, filtration of the Wine

After fermentation, wine samples were filtered by using muslin cloth and racked to settle down the cell biomass and other debris in it. Clarification of wine was done to remove insoluble matter suspended in the wine. After fermentation, wine samples were clarified by using gelatin (400mg/L) for clarification of wine sample. Centrifugation of wine was carried out by using ultra centrifuge at 5000 rpm for 20 minutes for purification of wine. Wine was filtered by using muslin cloth. After filtration, appropriate labels were given to pre-sterilized glass bottles and wine was stored in it. It was kept at room temperature for aging.

2.4 Analysis of the wine

2.4.1 pH

Determination of pH was carried out during the fermentation process by digital pH meter.

2.4.2 Total Soluble Sugars

An instrument was used to measure the refractive index of a liquid, which was used to measure sugar concentration in juice and wine sample (Ranganna, 1979) [10]. Hand refractometer of range 0 to 32 was used.

2.4.3 Titrable acidity

Titration Acidity was determined by Anonymous, AOAC method, (1970) [2].

2.4.4 Reducing sugar

3-5 Dinitrosalicylic acid (DNSA) is used extensively in biochemistry for the estimation of reducing sugars (Miller 1972). 100 µl of the sample was taken and the sugars were extracted with hot 80% ethanol twice (5 ml each time). The supernatant was collected and evaporated it by keeping it on a water bath at 80 °C. 10 ml water was added to dissolve the sugars. 3 ml of the extract was pipetted out in the test tubes. DNSA reagent (3ml) was added. Contents were heated in a boiling water bath for 5 min. When the contents of the tubes were still warm, 1 ml of 40% Rochelle salt solution was added. Tubes were cooled and the intensity of dark red color at 510 nm was noted down. Series of standards was run using glucose (0–500 µg/ml) and a graph was plotted. The amount of reducing sugars was calculated in the sample using the standard graph, Absorbance corresponds to 1 ml of test = x µg of glucose

10 ml contains = $x / 1 \text{ ml} \times 10 \text{ mg}$ of glucose = % of reducing sugars.

2.4.5 Specific Gravity

The specific gravity was measured using the following equation, (refer to the USDA Brix measurement doc. for the exact conversion numbers)

Specific Gravity = $1 + (0.004 \times \text{°Brix})$

2.4.6 Alcohol Content

The total alcohol of the wine samples was determined by the specific gravity method (Anonymous, AOAC, 2000) [3] using following formula

$$\text{ABV (\%)} = \frac{[1.05 \times (\text{OG} - \text{FG}) \div \text{FG}]}{0.79}$$

Where- OG is the initial specific gravity measurement of juice, FG is the final specific gravity measurement of wine and ABV is alcohol by volume.

2.4.7 Total phenolic compounds

A sample of 0.5 g was taken and dissolved in equal amount of water and ethanol. From the dissolved solution 0.2 ml was taken and made to 3ml with distilled water. Folin-ciocalteu reagent (FCR) of 0.5 ml was added and kept for 3 minutes. Sodium carbonate (20%) of 2 ml was added with sample solution and kept in boiling water bath for 1 min and the reading was obtained by observing absorbance at 650nm. (Jebittas, R and Allwin, J., 2016) [4].

2.4.8 Percent total sugar

The filtrate obtained in the estimation of reducing sugar was used. An aliquot of 50 ml, from the filtrate, was taken to which 5 ml of dilute hydrochloric acid (1:1) was added and the sample was left for inversion over night at room temperature. Then the solution was neutralized with 40 per cent sodium hydroxide till pink color appear using phenolphthalein as indicator and the final volume was made up to 100 ml with distilled water. The solution was titrated against boiling Fehling's mixture as described earlier. The percentage of total sugar was expressed as invert sugars according to following formula:

$$\text{Total Sugar (\%)} = \frac{\text{Glucose equivalent (0.05) X Total Volume made up X Volume made after inversion}}{\text{Titer X Weight of sample}}$$

2.4.9 Ascorbic acid (Vit-C)

Ascorbic acid content was estimated by the method of Klein *et al.* (1982) [5].

Reagents

- Oxalic acid: 4%
- Dye solution: 42 mg of sodium bicarbonate and 52 mg of 2, 6-dichlorophenol indophenol were mixed in 200 ml of distilled water.
- Stock standard solution: 100 mg ascorbic acid was dissolved in 100 ml of 4 % oxalic acid solution in a flask (1 mg/ml).
- Working standard solution: 10 ml of the stock solution was diluted to 100 ml with 4 % oxalic acid and the concentration of this working solution was taken 100µg/ml. 5 ml of the working solution was pipetted into 100 ml of conical flask followed by 10 ml of 4 % oxalic acid was added and titrated against the Qdye solution (V1 ml). End point was determined by the appearance of pink color which was persisted for a few minutes. 10 ml of sample (juice or wine) was taken and 100 ml of volume was made with 4 % oxalic acid solution. 5 ml of this supernatant was added to 10 ml of 4 % oxalic acid and titrated against the dye (V2 ml). The results thus obtained were expressed in terms of mg ascorbic acid/100 ml of juice. Vit-C can be calculated by the formula,

$$\text{Ascorbic acid (mg/100 g sample)} = \frac{0.5 \text{ mg} \times V2 \times 100 \text{ ml} \times 100}{V1 \times 15 \text{ ml} \times \text{Wt. of the sample}}$$

Where, V1 (ml) is the volume of dye used for the end point of standard

V2 (ml) is the volume of dye used for the end point of sample

2.4.10 Antioxidant activity

Antioxidant capacity was determined by DPPH (di phenyl picryl hydrazyl) method according Mau *et al.*, (2004) [6] method with some modifications. To the methanolic extract of sample, tris HCl buffer (pH 7.4) and 1 ml DPPH was added. The contents were mixed immediately and the degree of reduction of absorbance was recorded continuously for 30 min. at 517nm. Antioxidant capacity was calculated by according to following formula-

$$\text{Antioxidant activity (\%)} = \frac{[\text{Control absorbance (0min)} - \text{sample absorbance (30 min)}]}{\text{Control absorbance (0 min)}} \times 100$$

2.5 Physicochemical analysis of wine produced at optimized parameters using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

Production of wine produced using mahua flower extract and pomegranate fruit juice using the optimized parameters with two different strains was carried out. The parameters like temperature, pH, inoculums concentration, incubation period were maintained to the optimum and the physico-chemical analysis was done. The physicochemical characteristics like TSS, titratable acidity, total sugars, reducing sugars, alcohol content, ascorbic acid content, total phenols, antioxidant capacity were analyzed and the comparison was established between the results of wine produced using two different yeast strains in 2 blends.

2.6 Effect of aging on wine produced using mahua flower extract and pomegranate fruit juice produced at optimum parameters

The physicochemical parameters were analyzed after the 2 months of aging and the variation in physicochemical parameters was studied. The parameters TSS, pH, titratable acidity, alcohol content total sugars, reducing sugars, ascorbic acid content, total phenols and antioxidant activity were taken into consideration for analysis of wine after 2 months of aging for all the 2 blends.

2.7 Sensory Evaluation of Wine

Wine product was compared for color, flavor, taste, clarity and overall acceptability by a panel of 08 judges on a nine hedonic scale (Amerine *et al.* 1965) [1] where 0-4 denotes dislike, 5 denotes very poor, 6 denotes poor, 7 denotes good, 8 denotes very good and 9 denotes excellent.

2.8 Statistical Analysis of Wine

The completely randomized analysis of variance (ANOVA) was used to analyze the obtained data. Mean separation and comparison was done using SPSS version 16.0. Significance was accepted at $P < 0.05$ and results are expressed as mean \pm Standard deviation from the mean.

3. Results and Discussion

3.1 Physicochemical analysis of sample before fermentation

Variation in the physicochemical characteristics of mahua flower extract and pomegranate fruit juice before fermentation is shown in the table 3.1.

Table 3.1: Physicochemical analysis of samples before fermentation

Factors	Mahua flower extract	Pomegranate fruit juice
(pH)	4.6	5.1
TSS (⁰ Brix)	12	9
Titratable acidity (%)	0.11	0.27
Total sugars (%)	7.46	8.68
Reducing sugars (mg/ml)	1.02	1.37
Phenolic compounds (%)	0.43	0.45
Total sugars (%)	7.45	7.88
Ascorbic acid (mg/100ml)	3.15	3.49
Antioxidant compounds (%)	64.87	73.98

3.2 Optimization of fermentation parameters

The optimization study was carried out by using different combination of temperature, pH, inoculum concentration and incubation period of fermentation.

3.2.1 Temperature

The effect of temperature on production of wine by process of continuous fermentation using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215) was carried out at different temperatures 25°C, 30°C and 35°C on A and B wines.

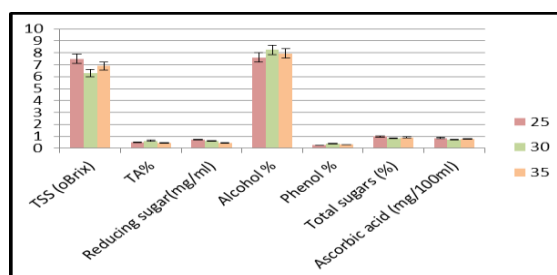


Fig 4.1: Effect of different temp. on A proportion wine by using yeast strain *S. cerevisiae* NCIM 3206

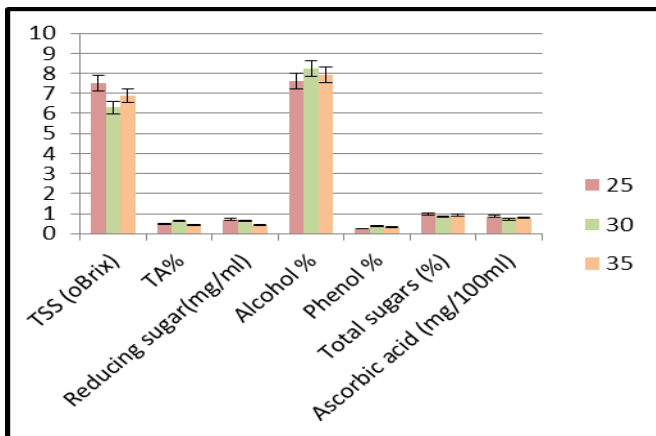


Fig 4.2: Effect of different temp. on B proportion wine by using yeast strain *S. cerevisiae* NCIM 3206

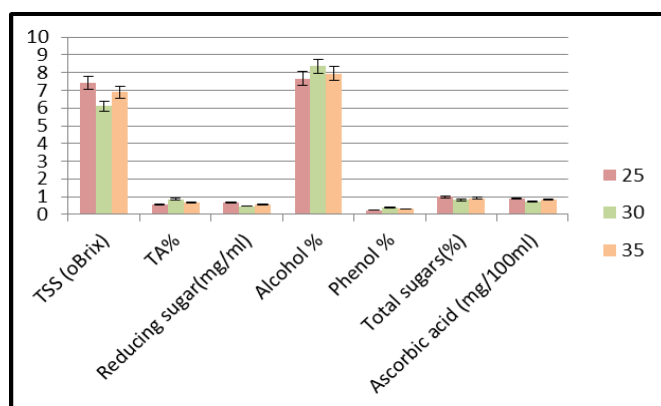


Fig 4.3: Effect of different temp. on A proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

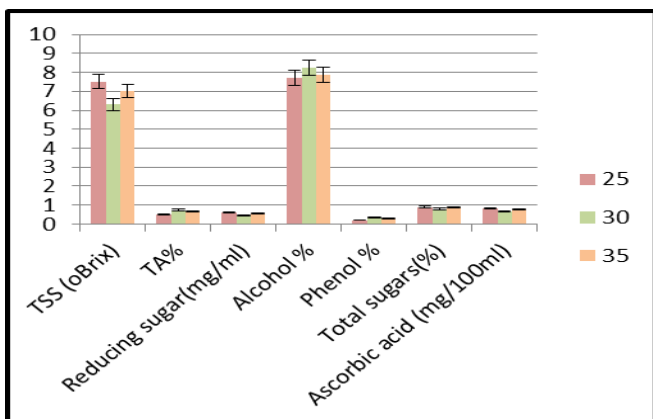


Fig 4.4: Effect of different temp. on B proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

3.2.1.1 Effect of different temperature on wine production of A and B proportions by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* NCIM 3215)

The fermentation process was shown to be prominent from 25 °C to 35 °C, being more distinguished at 30 °C.

Data in fig. 4.1.4.2, 4.3 and 4.4 shows the effect of temperature on wine production by using *S. cerevisiae* (NCIM 3206). It was clear from the data in table 4.3 that, maximum alcohol content (8.35%) was produced at 30 °C temperature compared to 25 °C and 35 °C. A significant steady increase in the alcohol content was observed in the wine produced using

the strain *S. cerevisiae* (NCIM 3206) from 25 °C to 30 °C and significant decline from 30 °C to 35 °C.

Hence, temperature at 30 °C was found most suitable for wine production of mixed *mahua* flower extract and pomegranate fruit juice by using *S. cerevisiae* (NCIM 3206). Satisfactory results by strain NCIM 3215 were obtained at 30°C. Hence, temperature of 30 °C was more suitable for wine production of mixed fruit by using *S. cerevisiae* (NCIM 3215).

S. cerevisiae NCIM 3215 exhibited better physicochemical characteristics compared to *S. cerevisiae* NCIM 3206 at 30°C for same level of substrate. Maximum alcohol content and antioxidant compounds were produced by *S. cerevisiae* NCIM 3215 compared to *S. cerevisiae* NCIM 3206. The fig 4.1, fig 4.2, fig 4.3 and fig 4.4 show that the higher percent of alcohol (8.40%) was produced by *S. cerevisiae* NCIM 3215 as compared to *S. cerevisiae* NCIM 3206. Hence, it was concluded that *S. cerevisiae* NCIM 3215 was more efficient than *S. cerevisiae* NCIM 3206 for mixed fruit wine production at 30°C with the same level of substrate.

3.2.2 pH

The effect of pH on wine production by process of continuous fermentation using *S. cerevisiae* NCIM 3206 and *S. cerevisiae* NCIM 3215 was carried out by varying the pH from 4.5, 5.5 and 6.5.

3.2.2.1 Effect of pH on wine production of A and B proportion by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* NCIM 3215)

The pH 4.5 significantly differed from pH 5.5 and pH 6.5 with respect to alcohol content and other parameters; with increase in pH above 4.5, the alcohol content reduced significantly because yeast produces acid rather than alcohol. So, pH 4.5 was considered optimum for production of wine and selected for further studies.

The *S. cerevisiae* (NCIM 3215) exhibited better physicochemical characteristics compared to *S. cerevisiae* (NCIM 3206) at pH 4.5 for the same level of substrate. Maximum alcohol content was produced by *S. cerevisiae* (NCIM 3215) as compared to *S. cerevisiae* (NCIM 3206) and it was represented graphically in fig. 4.5, 4.6, 4.7 and fig. 4.8. Hence, it was concluded that *S. cerevisiae* (NCIM 3215) is more suitable than *S. cerevisiae* (NCIM 3206) for mixed extract of *mahua* flower with pomegranate fruit juice wine production at pH 4.5 with the same level of substrate.

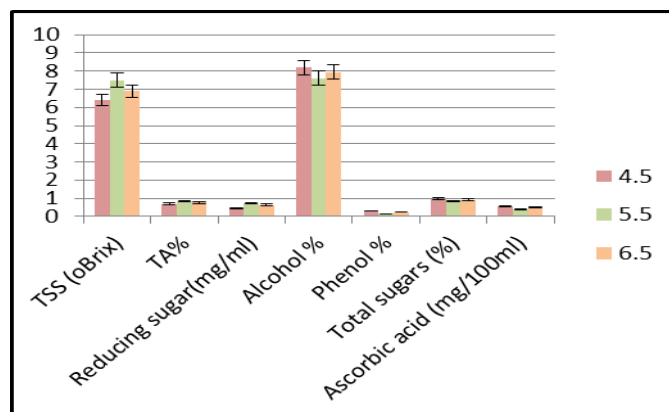


Fig 4.5: Effect of different pH on A proportion wine by using yeast strain *S. cerevisiae* NCIM 3206

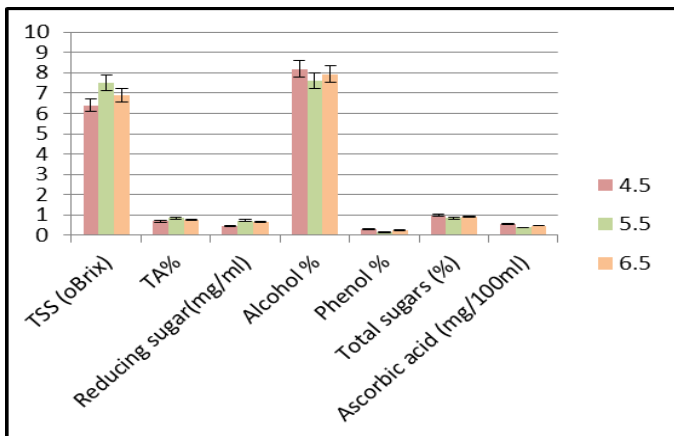


Fig 4.6: Effect of different pH on B proportion wine by using yeast strain *S. cerevisiae* NCIM 3206

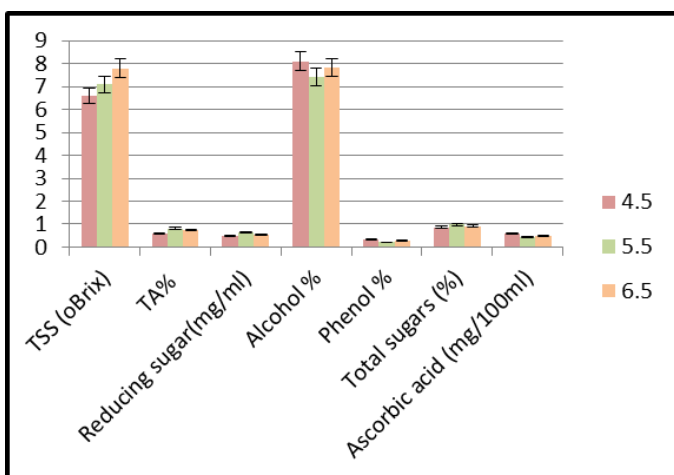


Fig 4.7: Effect of different pH on A proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

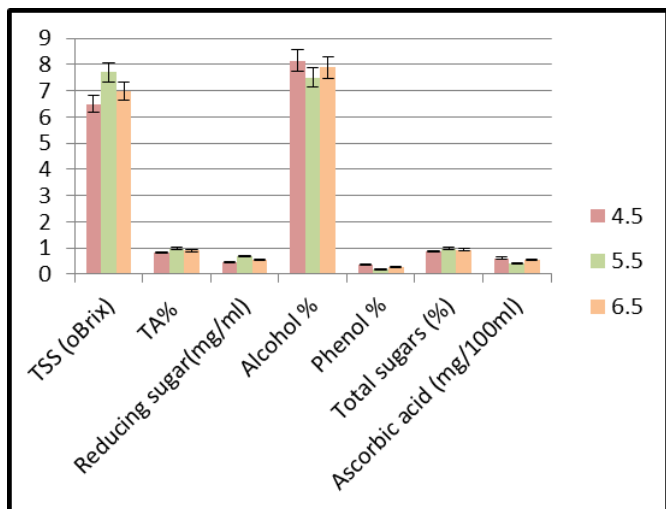


Fig 4.8: Effect of different pH on B proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

4.3.3 Inoculum concentration

4.3.3.1 Effect of inoculum concentration on wine production of A and B proportions by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

It indicated that inoculum concentration of 6% produced higher alcohol content as compared to 3% and 9% and inoculum concentration of 6% showed significant difference compared to 3% and 9%, with respect to alcohol content by *S. cerevisiae* (NCIM 3206).It can be concluded fig 4.9, fig. 4.10,

fig. 4.11 and fig. 4.12 that *S. cerevisiae* (NCIM 3215) exhibited better physicochemical characteristics compared to *S. cerevisiae* (NCIM 3206) at inoculum concentration of 6% for the same level of substrate. Maximum alcohol content was produced by *S. cerevisiae* (NCIM 3215) compared to *S. cerevisiae* (NCIM 3206). Hence it was concluded that *S. cerevisiae* (NCIM 3215) was more suitable than *S. cerevisiae* (NCIM 3206) for mixed extract of *mahua* flower and pomegranate fruit juice wine production at inoculum concentration of 6% with the same level of substrate.

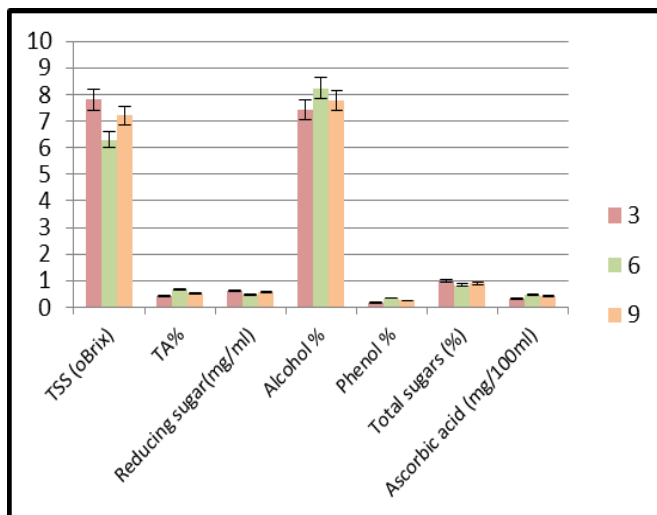


Fig 4.11: Effect of different inoculum concentration on A proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

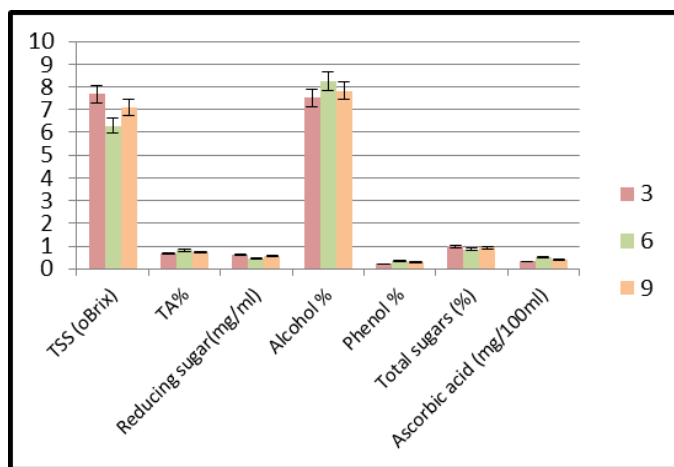


Fig 4.12: Effect of different inoculum concentration on B proportion wine by using yeast strain *S. cerevisiae* NCIM 3215

4.3.4 Incubation period

4.3.3.1 Effect of incubation period of starter culture on wine production of A and B proportions by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

From the fig. 4.13, 4.14, 4.15 and 4.16, it was concluded that 24 hour incubation period was suitable for wine production from mixed extract of *mahua* flower and pomegranate fruit juice by using *S. cerevisiae* (NCIM 3206) and selected for further studies.

Hence it was concluded that *S. cerevisiae* (NCIM 3215) was more efficient than *S. cerevisiae* (NCIM 3206) for mixed extract of *mahua* flower and pomegranate fruit juice wine production with 24 hr incubation period with the same level of substrate.

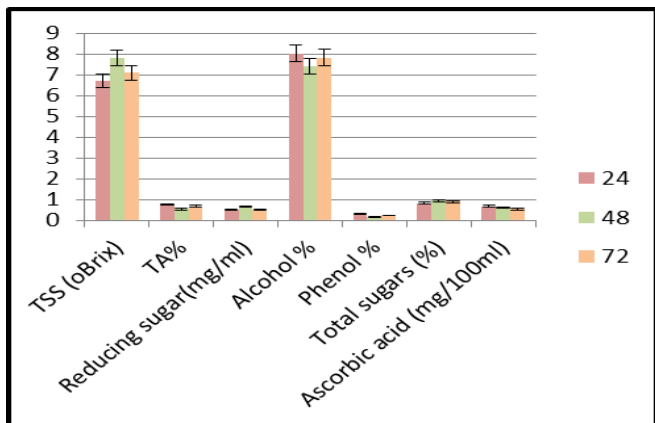


Fig 4.13: Effect of different incubation period on A proportion wine by using yeast *S. cerevisiae* strain NCIM 3206

4.4 Physicochemical analysis of A and B proportion wine samples produced at optimized parameters using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

From the results it was concluded that the *S. cerevisiae* (NCIM 3215) strain shown better physicochemical results than *S. cerevisiae* (NCIM-3206) at optimum fermentation parameters.

Table 4.1: Physicochemical analysis of A and B proportion wine samples produced at optimized parameters using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

Factors	A wine (NCIM 3206)	B wine (NCIM 3206)	A wine (NCIM 3215)	B wine (NCIM 3215)
TSS (°Brix)	7.1	6.9	6.9	6.7
TA%	0.72	0.79	0.75	0.82
Reducing sugar(mg/ml)	0.59	0.46	0.55	0.41
Alcohol %	7.82	7.93	7.93	8.03
Phenol %	0.22	0.32	0.27	0.36
Total sugars (%)	0.68	0.75	0.71	0.78
Ascorbic acid (mg/100ml)	0.37	0.28	0.40	0.32
Antioxidant (%)	74.84	81.53	77.47	84.18

(A wine – 10:90, B wine – 20:80 proportion)

The results of table 4.1 represents the physicochemical analysis of wine produced by using optimum fermentation parameters such as 30°C temperature, 4.5 pH, 6% inoculums concentration and 24hrs of starter culture incubation period for both the yeast strains *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215).

From the above mentioned results it was concluded that the *S. cerevisiae* (NCIM 3215) strain shown better physicochemical results than *S. cerevisiae* (NCIM-3206) at optimum fermentation parameters.

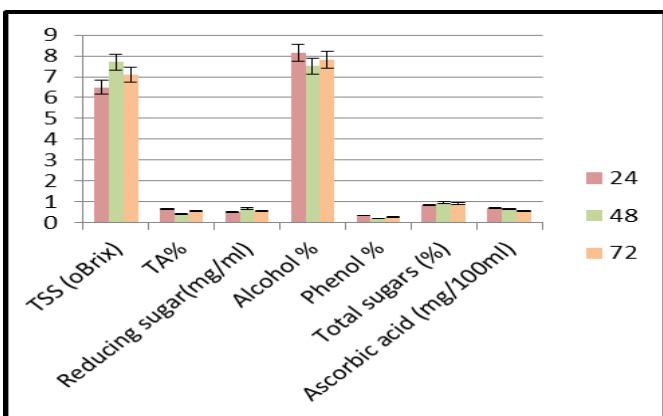


Fig 4.14: Effect of different incubation period on A proportion wine by using yeast *S. cerevisiae* strain NCIM 3206

4.5 Sensory evaluation

The wine produced by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215), with different fermentation parameters were evaluated for sensory parameters like color, flavor, taste, clarity and overall acceptability by a panel of 20 judges on a nine point hedonic scale.

4.6.1 Sensory evaluation of the A and B proportion wine samples produced using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215)

Wine produced by using *S. cerevisiae* (NCIM 3206) was acceptable with respect to sensory parameters like color, flavor, taste, clarity and overall acceptability as produced the wine exhibited good sensory and biochemical characteristics. Wine produced by using *S. cerevisiae* (NCIM 3215) was acceptable with respect to sensory parameters like color, flavor, taste, clarity and overall acceptability as it exhibited good sensory and biochemical characteristics in all the four wines.

5. Conclusion

5.1 Wine produced from mahua flower extract with pomegranate juice using *S. cerevisiae* (NCIM 3215) and *S. cerevisiae* (NCIM 3206)

Two different blends namely A (10:90) and B (20:80) were formulated using different concentrations of mahua flower extract with pomegranate fruit juice.

Temperature at 30 °C produced the higher level of alcohol, whereas it was slight lower at 25 °C and 35 °C among 2 proportions. It was concluded that temperature of 30 °C more suitable for wine production of *mahua* flower extract and

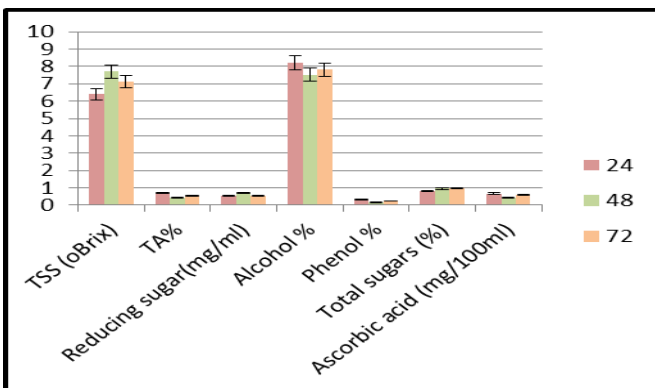


Fig 4.15: Effect of different incubation period on A proportion wine by using yeast *S. cerevisiae* strain NCIM 3215

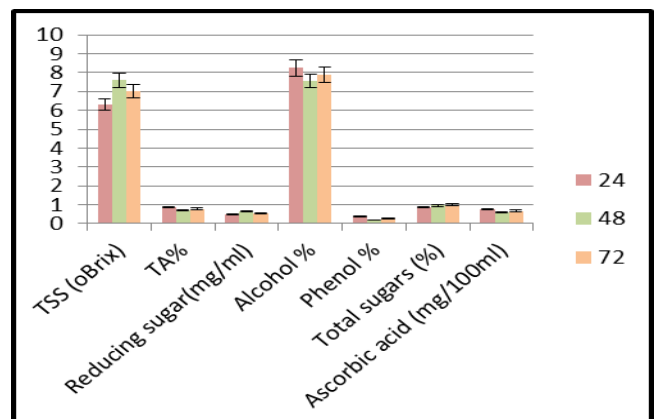


Fig 4.16: Effect of different incubation period on B proportion wine by using yeast *S. cerevisiae* strain NCIM 3215

pomegranate fruit juice by using *S. cerevisiae* (NCIM 3215) compared to *S. cerevisiae* (NCIM 3206).

The results revealed that 4.5 pH produced maximum alcohol content compared to 5.5 and 6.5pH for mixed mahua flower extract and pomegranate fruit juice wine production. It was observed that pH 4.5 was more suitable for wine production of mixed mahua flower extract and pomegranate fruit juice by using *S. cerevisiae* (NCIM 3215) as compared to *S. cerevisiae* (NCIM 3206).

The study concluded that inoculum concentration 6% produced maximum alcohol content compared to 3% and 9% for mixed mahua flower extract and pomegranate fruit juice wine production within 2 proportions. It was also observed that 6% inoculum concentration more suitable for wine production of mixed (mahua flowers and pomegranate fruit juice) by using *S. cerevisiae* (NCIM 3215) compared to *S. cerevisiae* (NCIM 3206).

The result showed that incubation period of 24 hrs old starter culture showed maximum alcohol production compared to 48 hrs and 72 hrs old starter cultures. Further It was also observed that incubation period of 24 hrs old starter culture was more suitable for wine production of mixed mahua flower extract and pomegranate fruit juice by using *S. cerevisiae* (NCIM 3215) as compared to *S. cerevisiae* (NCIM 3206).

From the results, it was concluded that the *S. cerevisiae* (NCIM 3215) strain shown better physicochemical results than *S. cerevisiae* (NCIM-3206) at optimum fermentation parameters for A and B proportion wine samples. The results of physicochemical parameters of 2 proportion wine samples after the storage of 2 months revealed that the strain NCIM-3215 reported better performance than strain NCIM-3206.

Wine produced was acceptable with respect to sensory parameters like color, flavor, taste, clarity and overall acceptability as the produced wine exhibited good sensory and biochemical characteristics. Sensory evaluation revealed that wine produced by using *S. cerevisiae* (NCIM 3206) and *S. cerevisiae* (NCIM 3215) were acceptable with respect to sensory and biochemical characteristics. Depending on the color, taste and overall acceptability, blend B (20:80) was selected as the most appreciable blend produced using strain *S. cerevisiae* (NCIM 3215).

The strain *S. cerevisiae* (NCIM 3215) produced better results as compared to strain *S. cerevisiae* (NCIM 3206) in all the aspects. Thus, strain *S. cerevisiae* (NCIM 3215) was concluded to be efficient for the fermentation process of mahua flower extract and pomegranate fruit juice.

6. References

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