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Snehlata Tiwari
Research Scholar, Warner College of Food and Dairy Technology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, India

Sangeeta Shukla
Assistant Professor, Warner College of Food and Dairy Technology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad India

Correspondence
Snehlata Tiwari
Research Scholar, Warner College of Food and Dairy Technology, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, India

Influence of different yeast strains on physico-chemical and rheological properties of herbal wine

Snehlata Tiwari and Sangeeta Shukla

Abstract
Wine has been considered as safe and healthy drink, besides an important adjunct to the diet. The present work was carried out to develop process methodology for the production of Herbal wine from *Hibiscus rosa-sinensis*. Hibiscus petals extract supplemented with sugar proved to be a good medium for the growth of different Strains of *Saccharomyces cerevisiae* for making the Hibiscus wine. Effect of different yeast strains of *Saccharomyces cerevisiae,* viz. MTCC 178, MTCC 180, MTCC 786 Strains on the enological and sensory characteristics of Hibiscus wines was determined. Different yeast strains influenced the physico-chemical characteristics of the wines produced to a variable extent. The higher rate of fermentation was shown by MTCC 178, compared to MTCC 180 and MTCC 786. Out of various strains, MTCC 178 gave the highest efficiency along with higher ethanol yield. The MTCC 786 strain produced lowest ethanol in wine. TSS, pH, total sugar, ethanol and colour did not contribute to separation of yeasts. This study deals with the rheological proper-ties of herbal wine too. Amongst the three yeast strain screened for alcoholic fermentation of reconstituted Hibiscus petals extract, *Saccharomyces cerevisiae* MTCC 178 was found most potent strain. The optimal alcoholic fermentation of reconstituted Hibiscus extract by *Saccharomyces cerevisiae* MTCC 178 was recorded at 25 ^{0}C, initial pH of must 5.0 and total soluble solid 24^0 brix with an inoculum level of 10% (v/v). Wine is very important along with other vinification practices as it influenced the chemical and sensorial quality of wine.

Keywords: *Hibiscus rosa-sinensis*, wine, fermentation, ethanol, principal component analysis, yeast, *Saccharomyces cerevisiae*

1. Introduction

The *Hibiscus rosa-sinensis* belongs to the family Malvaceae. Traditionally flower can be used as anti asthmatic agents. Many chemical constitute such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamin, riboflavin, niacin, and antimicrobial substances are present in this flower (Zhao J, 2010). The plant has been used in Ayurveda as a remedy for many ailments and diseases such as fevers, blood vomiting, stomachic troubles, irritable geneto-urinary tract conditions, swellings, ulcers and for hair strengthening as well. it Controls diabetes, lowers hypertension or blood pressure, analgesic and has anti-microbial properties.

For thousands of years mankind is using plant sources to alleviate or cure illness. Novel chemical compounds synthesis from the plant active constituents, which are of potential use in medicine and other useful application. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population. Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc. *Hibiscus rosa sinensis* having different pharmacological activities such as anticancer, anti-inflammatory, chemoprotective, antidiabetic and wound healing activities of their different parts. Selection of proper yeast strains is one of the major factor in production of good quality wine. The ability of producing alcohol from sugar varies differentially depending upon the yeast strains. This depends upon the several characters of the strain like: alcohol tolerance, optimum pH and temperature, ability to ferment sugar, etc. The criteria for selection of yeast strains assist in the choice of yeasts that are able to improve the quality and consistency of wine. The role of *Saccharomyces cerevisiae* in wine making has been described earlier and selected strains of *S. cerevisiae* have given definitely better results than the spontaneous fermentation.

The composition and quality of wine has been found to be related closely with the yeast used. Further, different yeast strains have different effects on the fermentation pattern, and physico-chemical of wines. Strains of Saccharomyces cerevisiae yeast which are known to produce different volatile profiles have been commonly used for alcoholic fermentation. But there is lack of information on Hibiscus wine production especially dealing with the suitability of local Hibiscus cultivars for wine production, other yeast strains, optimization of conditions of fermentation and characterization of its wine produced (both chemical and physical factors for quality). Accordingly, these aspects were investigated and the results are reported in this communication.
Therefore, the objectives of this study were to investigate the effects of different strains of Saccharomyces cerevisiae to Hibiscus wines on physicochemical properties and influence of providing health claims on their sensory acceptance.

2. Materials and Methods

2.1 Hibiscus flower and wine yeast culture

The flower of Hibiscus rosa sinensis were employed in the study. KMS, Di ammonium phosphate were purchased from local market of Allahabad. Different types of Saccharomyces cerevisiae strains MTCC 786, MTCC 178, MTCC 180 were employed in the study. Three standard wine yeast strains of Saccharomyces cerevisiae strains MTCC 786, MTCC 178, MTCC 180 were collected from IMTECH, Chandigarh, India. Cane sugar was used to raise the TSS of the must. The chemicals employed in analysis were of analytical grade.

2.2 Fermentation

The musts were prepared by diluting the Hibiscus powder with water. The TSS of the must was raised to 24ºB.To the must 100 ppm of SO2 in the form of KMS was added. Fermentations were performed in 1.5 litre glass vessels at 25^{o}C. Fermentations were initiated by the addition of yeast inoculums of all the three yeast strains grown in separate must diluted appropriately. The respective cultures were added at the rate of 2% (v/v). The fermentations were conducted as per the details given earlier. During fermentation, the rate (ºB/24h) was measured by recording fall in degree Brix every day. After completion of fermentation, the wines were racked and after proper clarification, it was bottled keeping 2.5cm headspace. The bottled wines were pasteurized at 62.5ºC for 15 min. The wines were analyzed after maturation for a year in the bottles and before evaluation, the wines were blended to 16oB by addition of sugar syrup to make acceptable acid/sugar/alcohol blend as reported earlier. It was allowed to mix properly and then evaluation for sensory analysis was carried out.

2.3 Analyses

2.3.1 Physico-chemical analysis

The wines prepared were analysed for TSS (oB), total solids, pH, titratable acidity, Protein content, reducing sugars and ethanol. TSS was measured using Erma hand refractometer (0-32oB) and corrected for the temperature variation. total solids was done by gravimetric method The pH was measured with ELTOP 3030 pH meter, Titratable acidity (as per malic acid) was estimated by titrating a known aliquot of sample against N/10 NaOH solution. Protein content was estimated by kjeldhal method whereas the reducing sugars were estimated by Lane and Eynon's volumetric method. Ethyl alcohol in the finished wines was determined by the colorimetric appropriately.

2.3.2 Rheological analysis

Samples of herbal wine was subjected to rheological tests also. These tests consisted in deter-mination of viscosity, colour and brightness. Viscosity was estimated with the help of viscometer while colour and brightness was determined were based on measurements of absorbance at 420 nm and 520 nm respectively by spectrophotometric method by using a 1 mm cuvette. The standard curve was plotted considering the concentration against absorbance. It was determined in triplicate

2.3.3 Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using one way analysis of variance (ANOVA). Differences were determined using Fisher's Protected Least Significant Difference (LSD) with P ≤ 0.05. The results of sampling cases and species were plotted simultaneously in a graph to interpret the results. In the data, physico-chemical parameters were used as sampling units and the yeast as species.

3. Results and discussion

Table 1: Effect of different wine yeast strains on the Physico-chemical & Rheological characteristics of Herbal wine

Parameters	MTCC 178	MTCC 180	MTCC 786
Physico-chemical analysis			
Ethanol (%)	11.50	10.51	9.51
TSS (^{0} Brix)	16.38	15.46	14.40
Total Solids (%)	11.00	9.27	8.64
pH	4.62	4.34	3.36
Titrable Acidity (%)	0.50	0.61	0.78
Protein (%)	1.26	1.19	0.87
Reducing Sugar (%)	0.050	0.070	0.090
Rheological Analysis			
Viscosity (cp)	3.13	4.31	4.52
Colour (nm)	1.410	1.550	1.930
Brightness (nm)	1.489	2.833	3.470

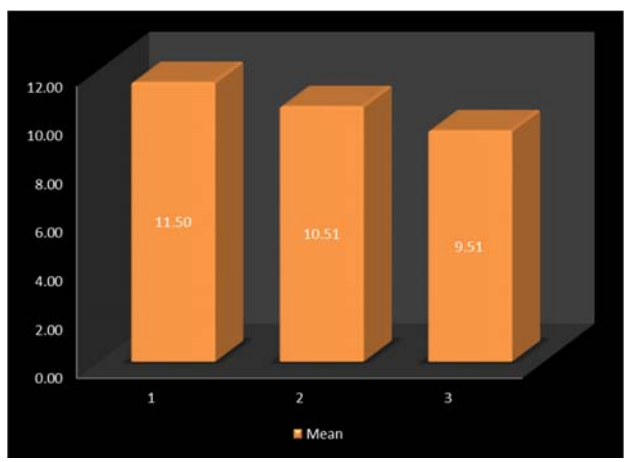

Fig 1: Fermentation efficiency of different yeast strains employed in Hibiscus wine preparation

The physico-chemical characteristics (Table1) of the Hibiscus wines prepared with different yeast strains clearly show that All the yeast strains recorded rate of fermentation higher than 1.6. Strain MTCC 178 had highest rate of fermentation. The yeast strain suitable for wine production should have high fermentability, tolerance to ethanol, sedimentation property and no effect on titratable acidity. Further, The Fig. 1 reveals that the strain MTCC 178 produced higher Alcohol (11.50 %) than other strains, which is of great interest to produce high quality wine. The amount of ethanol produced by the yeast is desirable character and from this point of view MTCC 178 proved to be the best. Further, all the three strains MTCC 180 and MTCC 786.

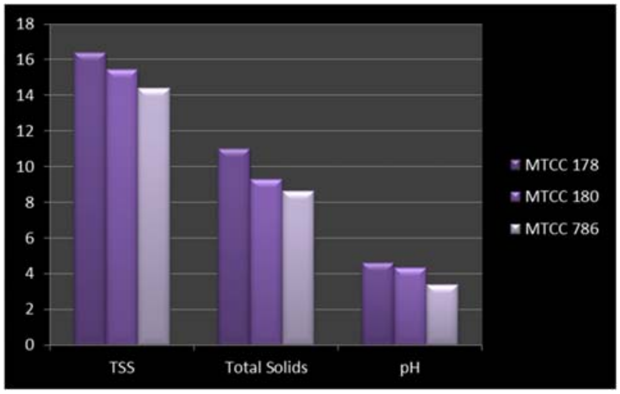

Fig 2: Effect of different yeast strains on TSS, Total solids and pH

TSS (Table 1) of all the wines ranged from 14.40 to 16.38 and all the wines were fermented to dryness as the residual concentrations of total sugars of all the wines was less than 0.5%. Although the The Fig. 2 reveals that the TSS of the wines (MTCC 178) was quite high, the residual sugar content was very low. It is because of the fact that TSS includes all soluble components including sugar. Total solids of all the wines ranged from 8.64 to 11.00 the pH (Table 1) of wines was according to their respective acidities. Here exists a correlation between PH and acidity of the sample. The higher the acidity, the lower the PH of the wine. A similar study conducted by Abbo *et al*., (2006) revealed that there is a corresponding reduction in pH as the acidity increased in sour wine. The pH of the wine was approx 4.00.

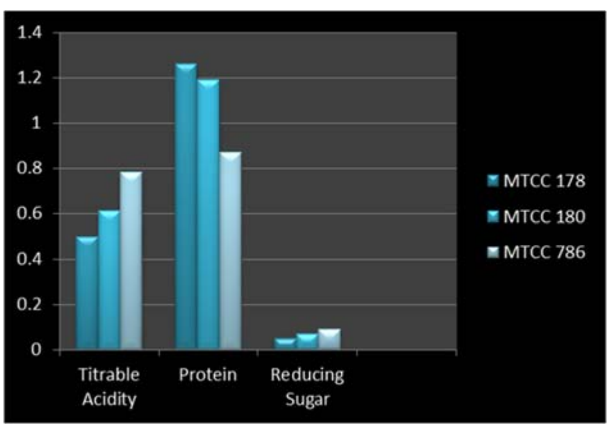

Fig 3: Effect of different yeast strains on titrable acidity Protein and Reducing sugar

The titratable acidity is an important characteristic varying between 0.50 to 0.78% (Table1) which was virtually comparable in all the wines. Thus, yeasts did not influence the acid production in the wines and is desirable. The protein content of the beverage ranged between 0.05-1. 00%. The low protein content may be attributed also to the effect of heat process involved in its extraction which might have destroyed some amino acids with resulting beverage (Oluwaniyi *et al*., 2009). However, the protein contents of the formulated wine (1.70-1.90)%.The reducing sugar was higher in wine MTCC 786 (0.090) as compare to MTCC 178 (0.050).Which shows that yeast strain MTCC 178 consumed more sugar.

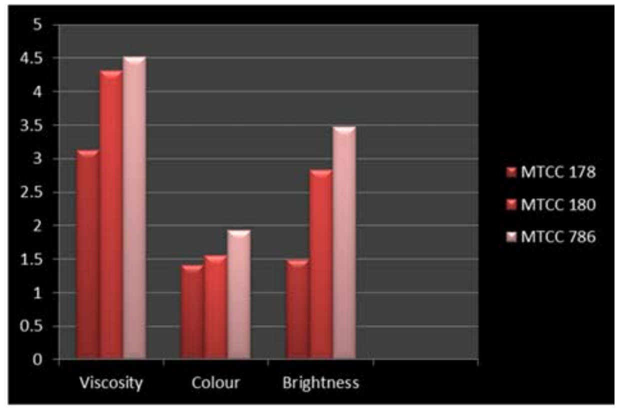

Fig 4: Effect of different yeast strains on viscosity Color and Brightness

Viscosity of wine MTCC 178 is mainly influenced by colloids. Colloids perform thermal motion (Brownian motion), gradually diffuse and settle, give rise to osmotic pressure, and participate in formation of gels. Wine (MTCC 786) contain relatively big amounts of thermolabile proteins. When the temperature increases above critical temperature, these proteins coagulate, hence viscosity increases, too (Monteiro *et al*., 2001) Fig 4 reveals that Color and brightness of the wine decreased significantly with difference in the inoculum level. The highest intensity of color and Brightness (Table 1) was (1.930) (3.470) respectively for MTCC 786.

4. Conclusions
The current work was aimed at exploring the vast alternatives of beneficial herbs and botanical ingredients to further enhance the efficacy and functionality of ever popular health beverage i.e. wine. It can be concluded that MTCC 178 strain is most suitable for herbal wine production and various yeast strains influenced the fermentation behavior, Physico-chemical and Rheological characteristics of the resulting wines. Our results are of great practical importance as the prepared herbal wines besides being a tasteful addition to food might also prove to be a health drink Thus, use of appropriate yeast strain for the preparation of Hibiscus wine is very important along with the other vinification. Further studies have been planned to assess the therapeutic potential of the prepared wines in a suitable murine model.

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