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EFFECT OF LEVELS OF YEAST AND SUGAR CONCENTRATIONS ON ETHANOL PRODUCTION AND BIO-CHEMICAL CHANGES OF HIBISCUS FLOWERS WINE

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ABSTRACT

The experiment was carried out at Post-Harvest Technology Laboratory, Section of Horticulture, College of Agriculture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during the year 2023-2024. Experiment was laid out in a Factorial Completely Randomized Design (FCRD) with two factors, as factors 'A' constitute levels of yeast (*Saccharomyces cerevisiae* var. ellipsoideus inoculated at 20, 30 and 40 ml/lit) and factor 'B' constitutes levels of TSS (20, 22, 24 and 26 ºBrix). Wine samples were evaluated for bio-chemical properties at fresh wine and after maturation period of seven months. Different levels of yeast and TSS and their interactions showed significant differences on hibiscus flowers wine. Findings revealed that wine prepared with *Saccharomyces cerevisiae* var. ellipsoideus inoculated at 40 ml/lit and TSS maintained at 22 ºBrix in must exhibited significantly superior results in respect of ethanol production (8.45 %) and TSS (6.49 ºBrix), pH (3.41) and titratable acidity (0.55) after seven months of maturation. The present study suggests that quality wine of hibiscus flowers can be prepared by must inoculated with yeast at 40 ml/lit and TSS at 22 ºBrix.

Keywords **:** Must, Fermentation, Red Wine, *Hibiscus rosa-sinensis*, *Saccharomyces cerevisiae* var. ellipsoideus.

Introduction

Flowers have medicinal properties and used in medicines in history of human civilizations. Pushpa Ayurveda is a special branch of ayurveda which gives details of using flowers in the treatment of diseases. Pushpa Ayurveda deals with preparation of rasayana medicines with 18000 kind of flowers (Vardhan, 1985). Hibiscus (*Hibiscus rosa-sinensis*) is a flowering shrub belonging to family Malvaceae and native to China (South East Asia). Hibiscus flower is rich in phytochemicals and nutrients hence it can be used in the value addition for production of beverages like wine. The flower contains many chemical constituents such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, and antimicrobial substances (Zhao *et al.* 2010). This flower is also high in anthocyanins and organic acids such as citric acid, malic acid and tartaric acid (Alobo and Offonry, 2009). Wine is non-distilled alcoholic beverage and prepared by grape and other fruits since long time, likewise fruits wine can be prepared from flowers also. Flowers like hibiscus, rose, calendula, lavender and mahua contains a typical taste, aroma and flavour and can be used in fermentation process for preparation of quality wine. The flower based fermented and un-distilled product; wine contains most of the nutrients present in

the original flower juice. The nutritive value of wine is increased due to the release of amino acids and other nutrients from yeast during fermentation (Amalu *et al.* 2019).

Materials and Methods

Fresh hibiscus (*Hibiscus rosa-sinensis*) flowers were collected and brought to the PHT laboratory for analysis

The laboratory experiment was conducted at Post-Harvest Technology Laboratory, Section of Horticulture, College of Agriculture, Dr. PDKV, Akola, during the year 2023-2024. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with two factors, twelve treatment combinations and three replications. Factor 'A' consists three levels of yeast (*Saccharomyces cerevisiae* var. ellipsoideus inoculum at 20, 30 and 40 ml per litre) and factor 'B' consist of four levels of TSS (20, 22, 24 and 26 ºBrix). The observation of fresh and seven months maturated wine with respect to ethanol, TSS, pH and titratable acidity were recorded.

The wine prepared from hibiscus flowers was analyzed for physico-chemical characteristics by standard methods described by A.O.A.C. (2000). Ethanol content of wine was estimated by using specific gravity method (Ranganna, 1979). This method provides an approximation of the alcohol content only. The Total Soluble Solids (TSS) was determined by using Atagomake RX 1000 digital refractometer, pH of wine was determined with help of digital pH meter (Model 411). The method described by Ranganna (1979) was adopted for the determination of titratable acidity of wine, in which one gram sample was diluted with 10 ml of distilled water and then filtered the solution. Then filtered solution was titrated against the standard NaOH (0.1N) solution, using phenolphthalein as an indicator. The appearance of light colour considered the end point of titration. Therefore, study was undertaken to study the effect of yeast and TSS levels on ethanol production and bio-chemical changes of hibiscus flowers wine.

Procedure for wine making: The procedure for wine making was thus carried out with slight modification in general method described by (Bhagwat *et al.* 2023).

Flow chart for preparation of hibiscus flowers wine.

Fresh hibiscus flowers
↓
Pulp preparation
↓
Dilution of pulp with water (1:4)
↓
Maintaining TSS as per treatment
↓
Amelioration of must with DAHP (0.1%)
↓
Addition of citric acid (0.5%)
↓
Addition of sodium benzoate (200 ppm)
↓
Inoculation with yeast culture as per treatment after 4 hours
↓
Fermentation
↓
After complete fermentation addition of Bentonite (1%)
Siphoning
↓
Bottling and corking
↓
Storage and Aging (maturation)

Results and Discussion

The data presented in Table 1 and 2 in respect of ethanol production and TSS, pH and titratable acidity of fresh and 7 months maturated wine exhibited significant differences due to different treatments of yeast and TSS and their interactions.

Ethanol

For fresh and 7 months of maturation of hibiscus flowers wine, due to effect of levels of yeast Y_3 (40 ml/l) recorded significantly maximum ethanol content (8.00 % and 8.36 %, respectively). However, minimum ethanol content (7.24 % and 7.49 %, respectively) was recorded in Y_1 (20 ml/l). In terms of different levels of TSS significantly maximum ethanol content (7.84 % and 8.20 %, respectively) was recorded in T_2 (22 ºBrix) and minimum ethanol content (7.58 % and 7.90 %, respectively) was recorded in T_4 (26 ºBrix). Due to interaction effect of yeast and TSS levels significantly maximum ethanol content (8.05 % and 8.45 %, respectively) was recorded with Y_3T_2 (Yeast inoculum at 40 ml/l and TSS 22 ºBrix). Whereas, maximum TSS (7.04 % and 7.30 %, respectively) was recorded with Y_1T_4 (Yeast inoculum at 20 ml/l and TSS 26 ºBrix).

From the results, it is apparent that, higher levels yeast shows early fermentation and moderate levels of TSS shows higher production ethanol. Hibiscus flowers must inoculated at 40 ml/lit and TSS maintained at 22 ºBrix converted more sugars into alcohol. Thus, it could be said that the optimum levels of inoculum for *Saccharomyces cerevisiae* var. ellipsoideus at 40 ml/lit and TSS maintained at 22 ºBrix for hibiscus flowers wine production. The present findings conformed with research work carried out by Sevada and Rodrigues (2011). Similarly, Hunbin *et al.* (2017), Tiwari *et al.* (2017), Gujar *et al*. (2023) and Bhagwat (2024) in rose petal wine production.

TSS

For fresh and 7 months of maturation of hibiscus flowers wine, due to effect of levels of yeast Y_3 (40 ml/l) recorded significantly minimum TSS (6.98 ºBrix and 6.70 ºBrix, respectively). However, maximum TSS (8.01 ºBrix and 7.84 ºBrix, respectively) was recorded in Y_1 (20 ml/l). Among different levels of TSS significantly minimum TSS (7.29 ºBrix and 7.05 ºBrix, respectively) was recorded in T_2 (22 ºBrix) and maximum TSS (7.62 ºBrix and 7.47 ºBrix, respectively) was recorded in T_4 (26 ºBrix). Due to interaction effect of yeast and TSS levels significantly minimum TSS (6.77 ºBrix and 6.49 ºBrix, respectively) was recorded with Y_3T_2 (Yeast inoculum at 40 ml/l and TSS 22 ºBrix). Whereas, maximum TSS (8.11 ºBrix and 8.04 ºBrix, respectively) was recorded with Y_1T_4 (Yeast inoculum at 20 ml/l and TSS 26 ºBrix).

From the results it was observed that there was a gradual decrease in TSS of hibiscus flowers wine during 7 months of maturation. The decrease in TSS might be due conversion of sugars into alcohol by yeast and also consumption of sugars by the action of yeast with the different levels of TSS. The results are in conformity with findings of various research workers, Tiwari *et al.* (2017) in hibiscus wine, Garg *et al.* (2014) in bael wine, Thakur *et al.* (2014) in pumpkin wine, Lenkannavar *et al.* (2015) in aonla wine, Belkhede (2019) in mahua wine, Gujar *et al.* (2023) in dried shoe flower and rose wine and Bhagwat (2024) in rose petal wine.

pH

For fresh and 7 months of maturation of hibiscus flowers wine, due to effect of levels of yeast significantly minimum pH (3.61 and 3.47, respectively) was recorded in Y_3 (40 ml/l). However, maximum pH (3.94 and 3.73, respectively) was recorded in Y_1 (20 ml/l). Among different levels of TSS significantly minimum pH (3.72 and 3.55, respectively) was recorded in T_2 (22 ºBrix) and maximum pH (3.83 and 3.64, respectively) was recorded in T_4 (26 ºBrix). Due to interaction effect of yeast and TSS levels significantly minimum pH (3.55 and 3.41, respectively) was recorded with Y_3T_2 (Yeast inoculated at 40 ml/l and TSS maintained at 22 ºBrix). Whereas, maximum pH (4.00 and 3.79, respectively) was recorded with Y_1T_4 (Yeast inoculated at 20 ml/l and TSS maintained at 26 ºBrix).

A slight decrease in pH was observed in hibiscus flowers wine during 7 months of maturation. The decrease in pH of hibiscus flowers wine during maturation could be attributed to precipitation of acids during fermentation. These findings are closer agreement with the research findings of research workers, Singh *et al.* (2013) in mahua wine, Tiwari *et al.* (2017) in hibiscus wine. Belkhede (2019) in mahua wine, Gujar *et al.* (2023) dried shoe flower and rose wine. Bhagwat (2024) in rose petal wine.

Table 1 : Effect of different levels of yeast and TSS on ethanol, TSS, pH and titratable acidity of hibiscus flowers wine.

Treatments	Parameters							
	Ethanol (%)		TSS (ºBrix)		pH		Titratable acidity (%)	
	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation
Factor 'A'	**Yeast levels**							
Y_1 (20ml/lit)	7.24	7.49	8.01	7.84	3.94	3.73	0.87	0.77
Y_2 (30ml/lit)	7.75	8.14	7.53	7.32	3.79	3.61	0.80	0.70
Y_3 (40ml/lit)	8.00	8.36	6.98	6.70	3.61	3.47	0.73	0.58
F Test	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**
SE(m) ±	0.004	0.006	0.003	0.004	0.005	0.004	0.002	0.003
CD at 5%	0.012	0.017	0.008	0.013	0.014	0.012	0.005	0.009
Factor 'B'	**TSS levels**							
T_1 (20 ºBrix)	7.60	7.92	7.61	7.37	3.80	3.62	0.80	0.70
T_2 (22 ºBrix)	7.84	8.20	7.29	7.05	3.72	3.55	0.78	0.65
T_3 (24 ºBrix)	7.63	7.97	7.50	7.27	3.77	3.59	0.80	0.67
T_4 (26 ºBrix)	7.58	7.90	7.62	7.47	3.83	3.64	0.82	0.71
F Test	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**
SE(m) ±	0.005	0.007	0.003	0.005	0.006	0.005	0.002	0.003
CD at 5%	0.014	0.020	0.009	0.015	0.016	0.014	0.006	0.010

Table 2 : Interaction effect of different levels of yeast and TSS on ethanol, TSS, pH and titratable acidity of hibiscus flowers wine

Treatment combinations	Parameters							
	Ethanol (%)		TSS (ºBrix)		pH		Titratable acidity (%)	
	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation	Fresh wine	7 Months of maturation
	Interaction effect							
Y_1T_1	7.13	7.35	8.06	7.79	3.92	3.71	0.86	0.76
Y_1T_2	7.67	7.94	7.88	7.67	3.89	3.70	0.84	0.75
Y_1T_3	7.12	7.35	8.01	7.89	3.96	3.74	0.90	0.77
Y_1T_4	7.04	7.30	8.11	8.04	4.00	3.79	0.91	0.80
Y_2T_1	7.73	8.13	7.64	7.41	3.82	3.63	0.81	0.71
Y_2T_2	7.80	8.22	7.23	6.99	3.72	3.55	0.78	0.66
Y_2T_3	7.77	8.17	7.50	7.30	3.76	3.59	0.80	0.68
Y_2T_4	7.71	8.06	7.76	7.61	3.85	3.66	0.82	0.73
Y_3T_1	7.95	8.27	7.13	6.92	3.68	3.51	0.75	0.63
Y_3T_2	8.05	8.45	6.77	6.49	3.55	3.41	0.71	0.55
Y_3T_3	8.00	8.39	6.99	6.62	3.59	3.45	0.72	0.57
Y_3T_4	7.98	8.34	7.01	6.77	3.63	3.48	0.74	0.59
F Test	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**
SE(m)±	0.008	0.012	0.005	0.009	0.010	0.008	0.003	0.006
CD at 5%	0.024	0.035	0.016	0.027	0.028	0.024	0.010	0.017

Titratable acidity

For fresh and 7 months of maturation of hibiscus flowers wine, due to effect of levels of yeast significantly minimum titratable acidity (0.73 % and 0.58 %, respectively) was recorded in Y_3 (40 ml/l). However, maximum titratable acidity (0.87 % and 0.77 %, respectively) was recorded in Y_1 (20 ml/l). In terms of different levels of TSS significantly minimum titratable acidity (0.78 % and 0.65 %, respectively) was recorded in T_2 (22 ºBrix) and maximum titratable acidity (0.82 % and 0.71 %, respectively) was recorded in T_4 (26 ºBrix). Due to interaction effect of yeast and TSS levels significantly minimum titratable acidity (0.71 % and 0.55 %, respectively) was recorded with Y_3T_2 (Yeast inoculated at 40 ml/l and TSS maintained at 22 ºBrix). However, maximum titratable acidity (0.91 % and 0.80 %, respectively) was recorded with Y_1T_4 (Yeast inoculated at 20 ml/l and TSS maintained at 26 ºBrix).

Titratable acidity gradually decreases during 7 months of maturation of hibiscus flowers wine. This might be due to the precipitation of different acids in terms of their salts. The results are in conformity with the findings of research workers on several wines. Tiwari *et al.* (2017) in herbal wine prepared from hibiscus, Belkhede (2019) in mahua wine, Sharma and Joshi (2003) in strawberry wine, Kumar *et al.* (2011) in custard apple wine, Lenkannavar *et al.* (2015) in aonla wine and Bhagwat (2024) in rose petal wine.

Conclusion

From the results, it is concluded that the hibiscus flowers wine prepared with different levels of yeast and TSS had a significant effect on ethanol production, TSS, pH and titratable acidity of wine. A thorough and effective fermentation process depends on determining the ideal yeast to TSS ratio, therefore, while preparing hibiscus flowers wine, it is advisable to maintain the TSS of must at 22 ºBrix and inoculate yeast at a concentration of 40 ml/lit for preparation of quality hibiscus flowers wine.

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