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Effect of different inoculum concentrations on physicochemical properties of wine produced from three different guava varieties

Yogendra Singh and Samsher

Abstract
Three different varieties of north Indian guava fruit including Allahabad Safeda, Chittidar and Punjab Pink were utilized for the wine processing using different inoculum concentrations of Saccharomyces cerevisiae IARI 1035 and native strains. Guava fruit wines were evaluated for TSS and pH and it was observed that both pH and TSS follow a decreasing trend with increase in the fermentation period. The total soluble solid content of the guava fruit juice was ameliorated to 20 degrees brix using sugar solution and was fermented for 30 days at 22±2 ºC. Lowest value of pH was found for guava wine made from Allahabad Safeda having 12% inoculum concentration of native strain while the highest pH was found for wine sample of Punjab Pink variety having 4% inoculum concentration of IARI strain at the end of fermentation period. We can conclude that the most desirable characteristics for the guava fruit wine production were seen with Punjab Pink variety of guava fruit S. cerevisiae IARI 1035 strain.

Keywords: wine, guava, TSS, Saccharomyces cerevisiae, inoculum concentrations

1. Introduction
Wine making from different fruits is an age-old practice and the possibility of wine making from different varieties of guava is explored in this study. There have been considerable increases in the consumption of fruit wine in the world during last few years. Alcoholic beverages, such as wine, beer, and liquor, have been part of human culture and development for 8,000 years. A non-alcoholic drink is one that contains little or no alcohol. This category includes low-alcohol beer, non-alcoholic wine, and apple cider if they contain less than 0.5% alcohol by volume. Wine (from Latin *vinum*) is an alcoholic beverage made from fermented grapes or other fruits. Due to the natural chemical balance, grapes ferment without the addition of sugars, acids, enzymes, water, or other nutrients. During the past few decades, grapes have been the main fruit that were used for wine production. Despite that, several studies have investigated the suitability of other fruits as substrates for the purpose of wine production (Joshi and Attri, 2005; Okunowo *et al.*, 2005) [7, 9]. Moreover, the non-availability and high cost of grapes, which is usually the fruit of choice for wine production in the tropical regions has necessitated the search for alternative fruit sources in tropical countries (Alobo and Offonry, 2009) [1].

In India, Guava is commonly called as poor man‘s apple widely naturalized in the country and is often considered as a “super fruit” due to its rich nutritional value. India is one of the major producers as well as exporter of guava to the developing and the developed world. This hardy fruit is cultivated widely all over India. The antioxidant properties in guavas are due to the presence of high amounts of vitamin C (Ascorbic acid) and a carotenoid lycopene (Celso *et al*., 2008) [2] which help in prevention of many degenerative diseases (Kadam *et al*., 2012) [8]. These fruits have a high digestive value, and also contain Vitamin A (beta carotene) and Vitamin C (ascorbic acid) in considerable amounts. The seeds are rich in omega-3 and omega 6 fatty acids, dietary fibers and mineral salts. Pleasant aroma and taste of guava are highly appreciated across India and make it competent in the market, either as guava juice or as mixtures with other juices or as guava wine.

Guava fruit undergo high rate wastage of fruits especially at their peak season of production. This necessitates the need for alternative method of preservation and post-harvest technologies towards value addition of guava that can not only reduce the level of post-harvest losses but also increase diversity of wines. Hence, the objective of the present study is assessing effects of different yeast inoculum concentrations on the physicochemical properties of the guava wine and conducting a comparison of the two strains of the yeast for the same.

2. Materials and Methods

Yeast strains used in the guava wine production

Yeast strain (*Saccharomyces Cerevisiae* 1035) was procured from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), PUSA, New Delhi. Guava sap obtained after washing the guava was inoculated on PDA media to obtain native strain of *Saccharomyces Cerevisiae* in laboratory, Department of Recombination Techniques, SVPUA&T, Meerut.

Media composition used for isolation of yeast strain

Potato Dextrose Agar (PDA), yeast extract and distilled water was used for isolation and maintenance of yeast strains.

Table 1.1: Characteristics of Three Varieties of Guava Fruit Used for Wine Production

S. No.	Variety	Location	Properties
1	Allahabad Safeda	Local market of Allahabad	Fruits are medium in size, white fleshed with good keeping quality; round in shape with few seeds.
2	Chittidar	Department of Horticulture, SVPUA&T, Meerut	Similar to the Safeda except that it has many pinkish red dots of the size of a pinhead on the surface of the fruit.
3	Punjab Pink	Local market of Punjab	Medium to large in size with attractive red coloured skin sometimes in summer season and golden yellow in winter season. The flesh is red having pleasant flavour.

Chittidar **Punjab Pink** **Allahabad Safeda**

Plate 1.1: Three Varieties of Guava Fruit Used for Wine

Isolation of Native Yeast Strain

Guava sap was taken and about 0.1ml of sap serially diluted and plated on a selective medium of potato dextrose agar (PDA). The inoculated plates were incubated for 48 hours at 25ºC. The colonies appeared were further purified. Pure colonies were isolated and tested for further characterization.

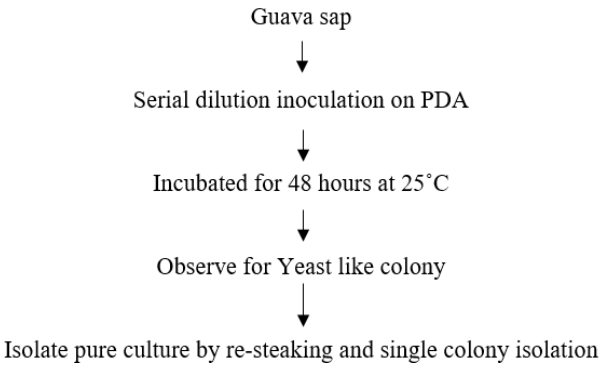

Fig 1.1: Flow Chart Showing Methodology for the Isolation of Yeast Strain

Preparation of Broth

30 gram YPD (1% yeast extract, 2% peptone and 2% dextrose) was mixed well with 600 ml distilled water and was heated for 5 minutes. 100 ml broth was poured in 250 ml conical flask and then autoclaved.

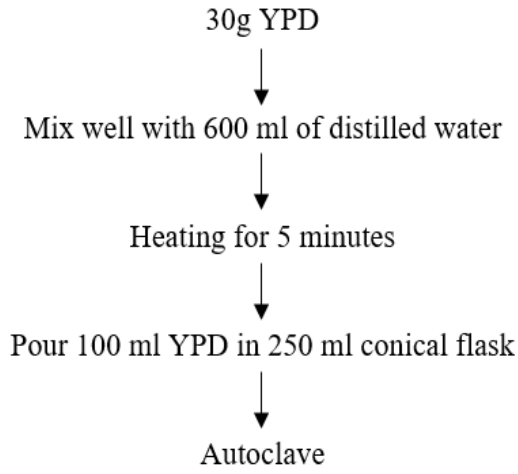

Fig 1.2: Flow Chart Showing Methodology for the Broth Preparation

Preparation of Yeast Culture

The inoculum of pure isolate of *S. cerevisiae* IARI strain and native strain were prepared in YPD broth for the fermentation where a loopful of slant culture was inoculated in 250 ml Erlenmeyer flasks containing 100 ml of YPD broth. It was incubated at 100 rpm and at 28±2˚ C for 24 hours to raise seed inoculum.

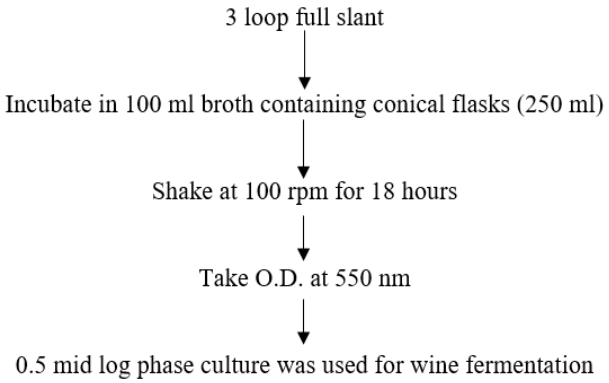

Fig 1.3: Flow Chart Showing Methodology for the Preparation of Starter Culture

Amelioration of TSS

Total Soluble Solid content (TSS) of enzyme treated guava juice was ameliorated to 20°B by adding sugar solution.

Adjustment of pH

The pH of the guava juice was adjusted to 4.0 by addition of calcium carbonate and citric acid respectively

Determination of Total Soluble Solids (TSS)

TSS (total soluble solid) of guava juice was measured by hand refractometer of range of 0-32° Brix and for measuring TSS of wine. Use of this method was recommended by (Srivastav and Kumar, 2009) [11] a brief description is given below.

A drop of sample was placed on the prism and the observation was taken in front of sunlight. The visible scale showed a dark line indication measuring TSS in degree Brix (°B).

Determination of pH

pH is the measurement of the logarithm of inverse of hydrogen ion concentration in the solution.

$$pH = -\log [H+]$$

Where, H+ = hydrogen ion concentration (g\ lit)

The electronic pH meter (Elico, LI -127) was calibrated using 7 pH and 4 pH standard buffer solutions. Then electrode was dipped in the test solution and the temperature knob was adjusted to temperature of test solution. The function selector switch was set to pH and reading of digital display was allowed to stabilize. pH values were determined with the help of electronic pH meter (Systronics µ pH system - 361) as recommended by Ranganna (2001) [10].

3. Results and Discussion

Tables 1.1 and 1.2 show physicochemical properties of guava wine obtained from fermentation of guava juice of three varieties *viz*. Allahabad Safeda (AS), Chittidar (CD) and Punjab Pink (PP) with different inoculum concentrations of *S. cerevisiae IARI 1035* and native strains. It was observed from Figures 1.4 to 1.9 that both pH and TSS follow a decreasing trend with increase in the fermentation period. These results reveal that TSS declines to nearly zero after 60 days in all the varieties of Guava for both *S. cerevisiae IARI* and native strains for all concentrations of inoculum used.

Results present in Tables 1.1 and 1.2 revealed that the wine sample of Allahabad Safeda having 12% inoculum concentration and native strain exhibited the lowest value of pH (3.29). However, the highest value of pH (3.48) of wine sample of Punjab Pink variety having 4% inoculum concentration and IARI strain was found after 60 days. However, in general the lower value of pH was found after 60 days in each wine samples of different varieties, inoculum concentration and strains. Overall *S cerevisiae* strain IARI performed better than native *S cerevisiae* strain and had higher fermentation efficiency over the native strain at all inoculum concentrations tested in this study *viz*. 4%, 8% and 12%.

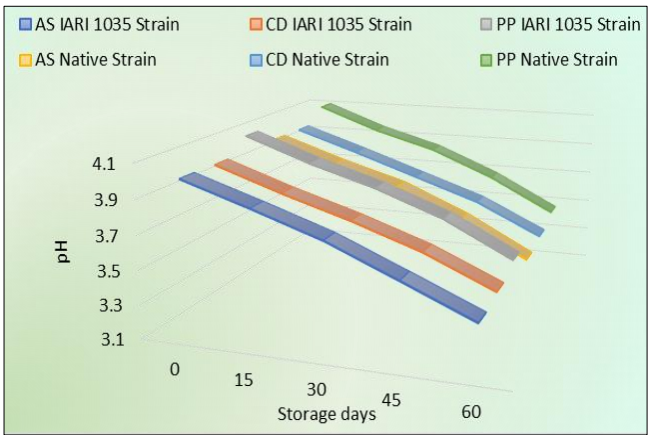

Fig 1.4: Variation in pH of guava wine with 4% inoculum concentration of *S. cerevisiae* IARI and Native strains

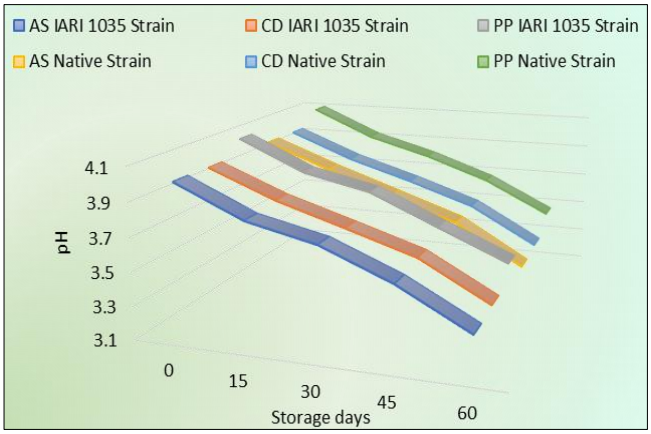

Fig 1.5: Variation in pH of guava wine with 8% inoculum concentration of *S. cerevisiae* IARI and Native strains

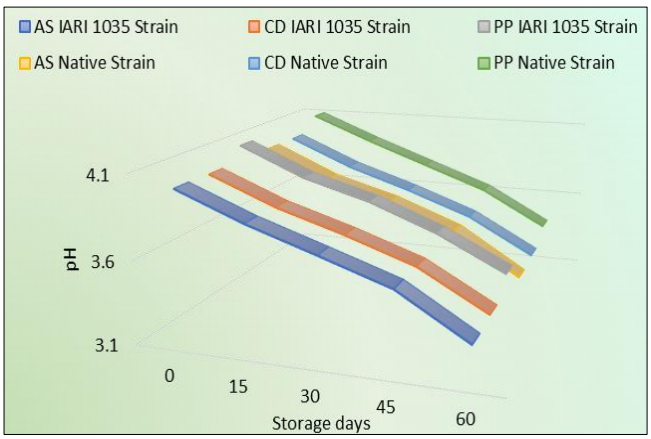

Fig 1.6: Variation in pH of guava wine with 12% inoculum concentration of *S. cerevisiae* IARI and Native strains

Table 1.1: Effect of inoculum concentrations on pH of guava wine samples

Storage Period (Days)	pH																	
	Inoculum Concentration						Inoculum Concentration						Inoculum Concentration					
	4%						8%						12%					
	IARI 1035 Strain			Native Strain			IARI 1035 Strain			Native Strain			IARI 1035 Strain			Native Strain		
	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP
0	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
15	3.87	3.86	3.96	3.89	3.87	3.95	3.82	3.84	3.92	3.85	3.89	3.91	3.84	3.83	3.92	3.82	3.80	3.94
30	3.74	3.74	3.84	3.75	3.73	3.84	3.73	3.71	3.83	3.71	3.72	3.81	3.70	3.71	3.81	3.71	3.69	3.79
45	3.56	3.60	3.69	3.58	3.60	3.67	3.56	3.58	3.65	3.57	3.58	3.67	3.56	3.56	3.65	3.55	3.54	3.64
60	3.39	3.42	3.48	3.36	3.40	3.46	3.33	3.35	3.47	3.33	3.35	3.46	3.31	3.33	3.44	3.29	3.32	3.41

Where, AS = Allahabad Safeda, CD = Chittidar, PP = Punjab Pink

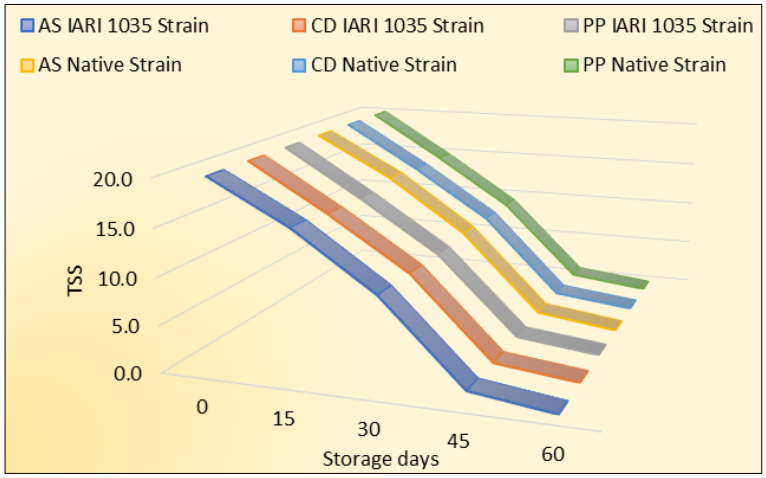

Fig 1.7: Variation in TSS of guava wine with 4% inoculum concentration of *S. cerevisiae* IARI and Native strains

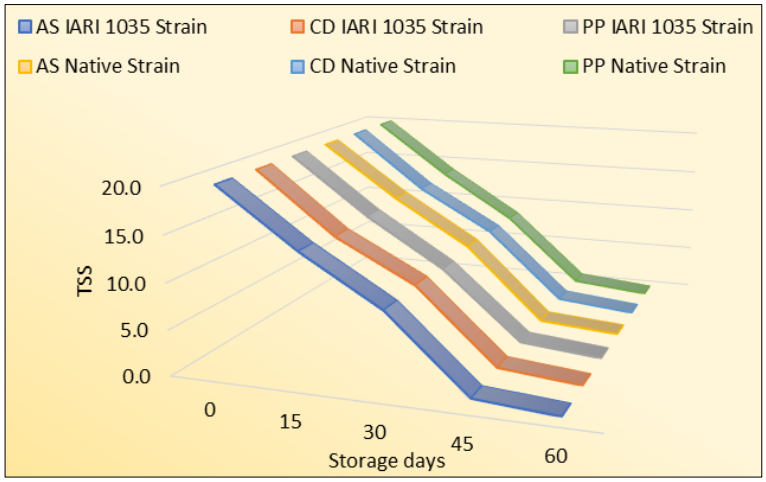

Fig 1.8: Variation in TSS of guava wine with 8% inoculum concentration of *S. cerevisiae* IARI and Native strains

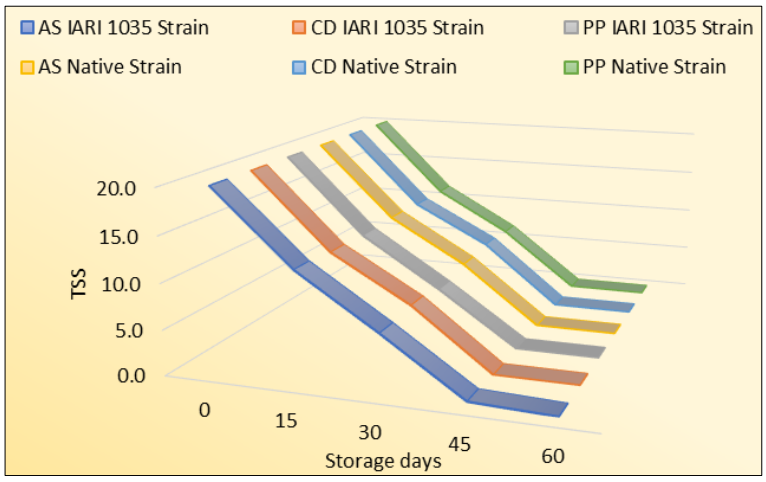

Fig 1.9: Variation in TSS of guava wine with 12% inoculum concentration of *S. cerevisiae* IARI and Native strains

Table 1.2: Effect of inoculum concentrations on total soluble solid (TSS) of guava wine samples

Total Soluble Solid (ºBrix)																		
Storage Period (Days)	Inoculum Concentration 4%						Inoculum Concentration 8%						Inoculum Concentration 12%					
	IARI 1035 Strain			Native Strain			IARI 1035 Strain			Native Strain			IARI 1035 Strain			Native Strain		
	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP	AS	CD	PP
0	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
15	15.50	15.09	14.93	15.70	14.90	14.73	13.83	13.33	13.67	14.00	13.50	13.83	12.00	11.66	11.50	11.70	11.52	11.50
30	9.67	9.50	9.33	9.47	9.42	9.16	8.67	8.83	8.33	8.53	8.61	8.50	6.33	6.67	6.00	6.67	7.00	6.67
45	1.17	1.03	1.90	1.12	1.01	1.03	0.67	0.93	0.9	0.67	0.8	0.83	0.33	0.20	0.08	0.01	0.92	0.02
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Where, AS = Allahabad Safeda, CD = Chittidar, PP = Punjab Pink

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