Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 2316-2321
Received: 12-06-2020
Accepted: 15-09-2020

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Assessing physicochemical properties of wine produced from three different guava varieties using different yeast concentrations and strains

Yogendra Singh and Samsher

Abstract
We have utilized three different varieties of north Indian guava fruit viz Allahabad Safeda, Chittidar and Punjab Pink for the wine processing. Different inoculum concentrations (4%, 8% and 12%) of Saccharomyces cerevisiae IARI 1035 and native strains were used for preparation of wine. Guava fruit wines were evaluated for acidity and ascorbic acid content and it was observed that acidity followed an increasing trend with increase in the fermentation period while ascorbic acid content followed. In general, higher value of acidity was observed after 60 days irrespective of varieties, inoculum concentrations and strains. We can conclude that the most desirable characteristics including acidity and ascorbic acid contents were observed for the guava fruit wine produced using Chittidar and Punjab Pink varieties of guava fruit with S. cerevisiae IARI 1035 strain.

Keywords: Wine, Guava, acidity, ascorbic acid, Saccharomyces cerevisiae, inoculum concentrations, IARI 1035

1. Introduction
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. 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. 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) [8, 11]. 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].

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. 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. 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) [3] which help in prevention of many degenerative diseases (Kadam *et al*., 2012) [9]. 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 physicochemical properties of guava wine produced from three different guava varieties different yeast concentrations and conducting a comparison of the two strains of the yeast for the same.

2. Materials and Methods

Yeast strains used in the production of guava wine

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.

IARI strain

Native strain

Fig 1: *S. Cerevisiae* (IARI and native strains) used in guava wine production

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.

Guava sap

↓

Serial dilution inoculation on PDA

↓

Incubated for 48 hours at 25˚C

↓

Observe for Yeast like colony

↓

Isolate pure culture by re-steaking and single colony isolation

Fig 2: 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.

30g YPD

Mix well with 600 ml of distilled water

↓

Heating for 5 minutes

↓

Pour 100 ml YPD in 250 ml conical flask

↓

Autoclave

Fig 3: 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.

3 loop full slant

↓

Incubate in 100 ml broth containing conical flasks (250 ml)

↓

Shake at 100 rpm for 18 hours

↓

Take O.D. at 550 nm

↓

0.5 mid log phase culture was used for wine fermentation

Fig 4: Flow Chart Showing Methodology for the Preparation of Starter Culture

Determination of Acidity

Acidity may be referred as the percent total acid in any food substance. The organic acid is responsible for the sourness of fruit. Acidity of various samples was determined by using the method as recommended by Ranganna (2001) [12]. A brief description is given below:

5 ml sample was dissolved in a 100 ml of distilled water and out of this 10 ml aliquot was taken and titrated with 0.1N NaOH using a few drops of phenolphthalein solution as indicator. The endpoint was denoted by the appearance of pink color. The titre value was noted and result was calculated as % acids using following equation:

$$\% \text{ Acidity} = \frac{\text{Vol. of NaOH} \times 0.1\text{N} \times 0.064 \times 100}{\text{Volume of sample taken} \times 1000} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Ascorbic Acid Estimation (Vitamin C)

Samples of wine were analyzed for the ascorbic acid (Vitamin C) content using 2.6- Dichlorophenol indophenol dye titrimetrically as per the AOAC's (Association of Official Analytical Chemists) official method **(AOAC, 1990)**.

A brief description of the ascorbic acid estimation procedure is given below:

1. 4% Oxalic acid
2. Dye solution: weigh 42 mg sodium bicarbonate into a small volume of distilled water. Dissolve 52 mg 2.6-dichlorophenol indophenol in it and make up to 200 ml with distilled water.
3. Stock standard solution: dissolve 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution in a standard flask (1mg/ml).
4. Working Standard: dilute 10 ml of the stock solution to 100ml with 4% oxalic acid. The concentration of working standard is 100 µg/ml.

Procedure

1. Pipette out 5 ml of the working standard solution into a 100 ml conical flask.
2. Add 10 ml of 4% oxalic acid and titrate against the dye (V1 ml). End point is the appearance of pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5 - 5 ml depending on the sample) in 4% oxalic acid and make up to a known volume (100 ml) and centrifuge.
4. Pipette out 5 ml of this supernatant, add 10 ml of 4%oxalic acid and titrate against the dye (V2 ml).

Calculation

$$\text{Ascorbic acid (mg/100 g)} = \frac{0.5 \text{ mg X } V_2 \text{ X } 100 \text{ ml}}{V_1 \text{ ml X } 15 \text{ ml X wt. of sample}} \quad (2.2)$$

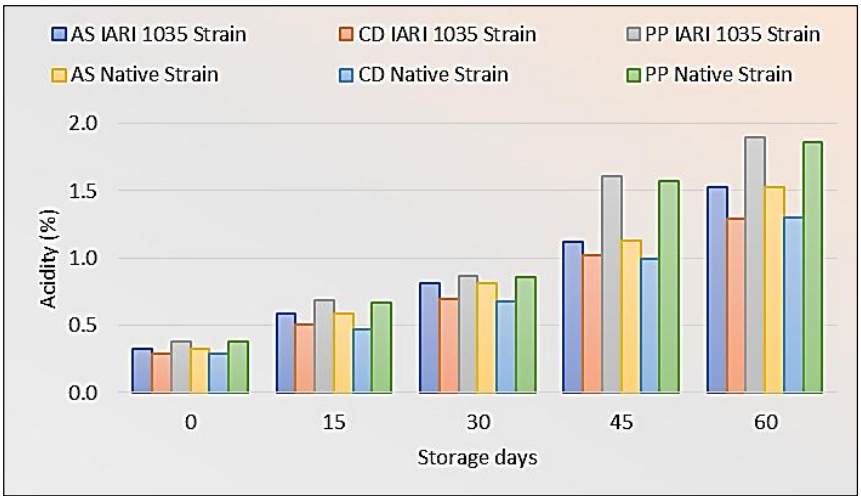

Fig 4: Variation in acidity of guava wine with 4% inoculum concentration of *S. cerevisiae* IARI and Native strains

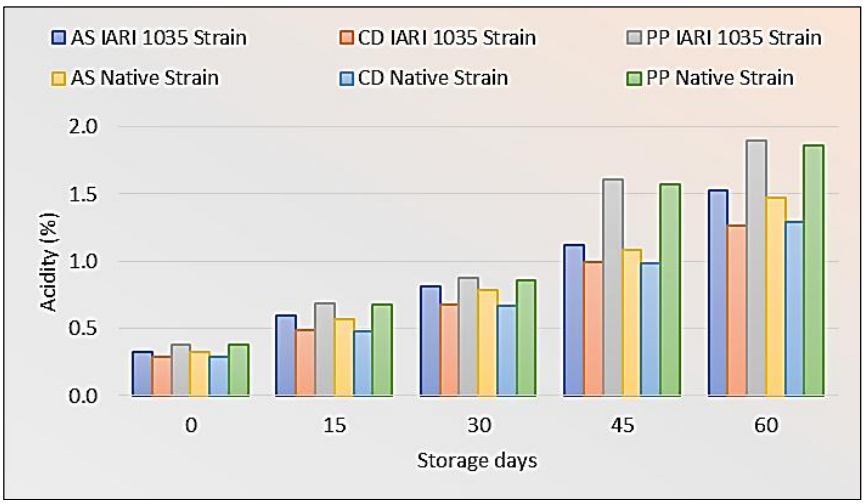

Fig 5: Variation in acidity of guava wine with 8% inoculum concentration of *S. cerevisiae* IARI and Native strains

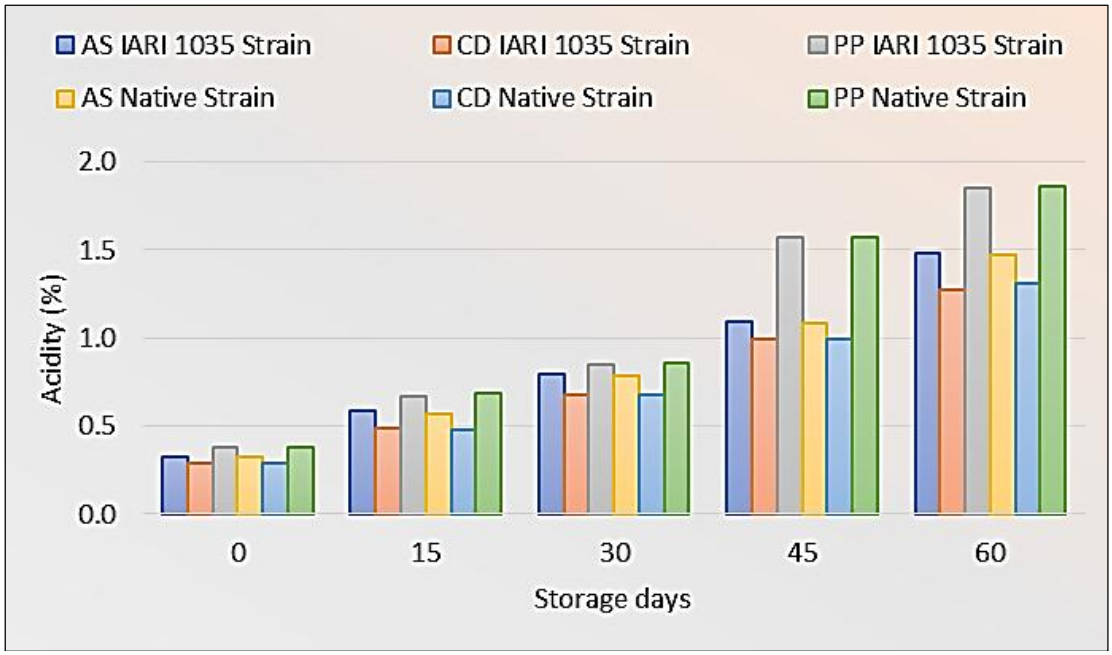

Fig 6: Variation in acidity of guava wine with 12% inoculum concentration of *S. cerevisiae* IARI and Native strains

Table 1: Effect of inoculum concentrations, strains and storage period on Acidity of guava wine samples

Acidity (%)																		
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	0.32	0.29	0.38	0.32	0.29	0.38	0.32	0.29	0.38	0.32	0.29	0.38	0.32	0.29	0.38	0.32	0.29	0.38
15	0.59	0.50	0.68	0.59	0.47	0.67	0.59	0.49	0.68	0.57	0.48	0.68	0.58	0.49	0.67	0.57	0.48	0.68
30	0.81	0.70	0.87	0.81	0.68	0.85	0.82	0.68	0.87	0.78	0.67	0.86	0.80	0.68	0.85	0.78	0.68	0.86
45	1.12	1.02	1.60	1.12	0.99	1.57	1.12	0.99	1.61	1.08	0.98	1.57	1.09	1.00	1.57	1.09	1.00	1.58
60	1.53	1.29	1.90	1.53	1.31	1.86	1.52	1.27	1.90	1.48	1.30	1.86	1.48	1.27	1.85	1.48	1.31	1.86

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

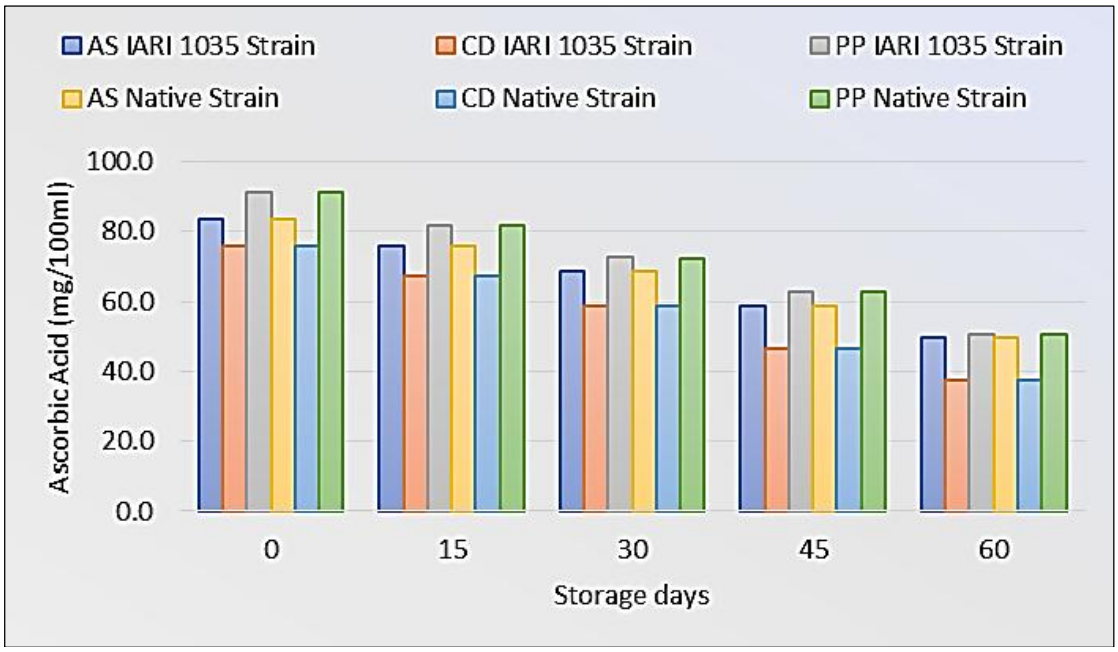

Fig 5: Variation in ascorbic acid content of guava wine with 4% inoculum concentration of *S. cerevisiae* IARI and Native strains

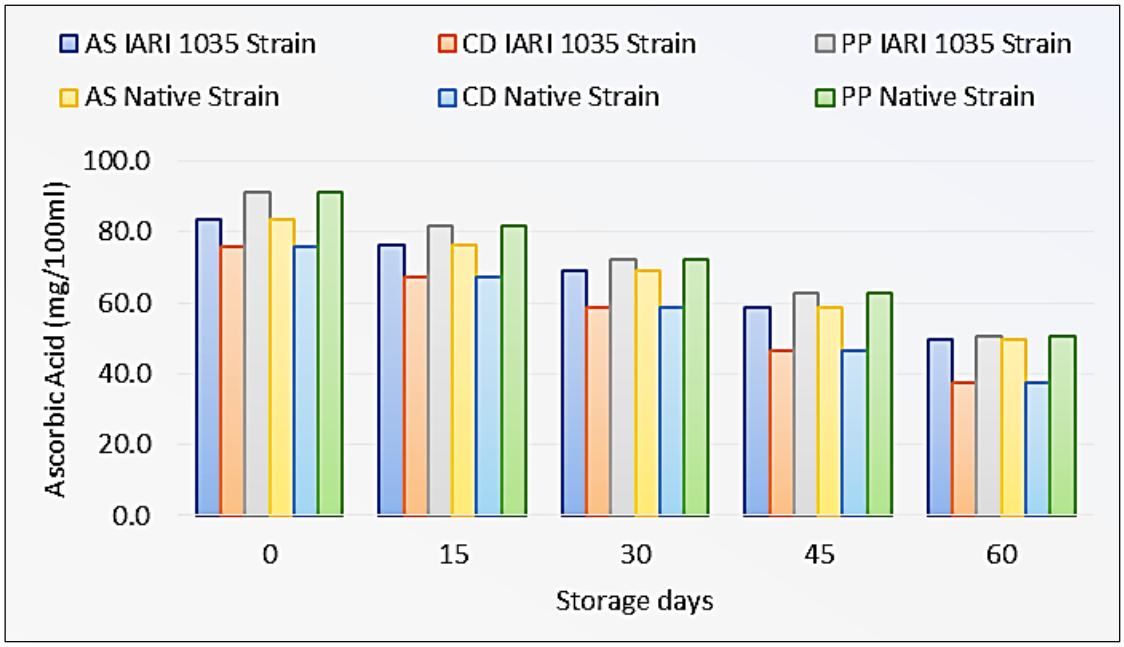

Fig 6: Variation in ascorbic acid content of guava wine with 8% inoculum concentration of *S. cerevisiae* IARI and Native strains

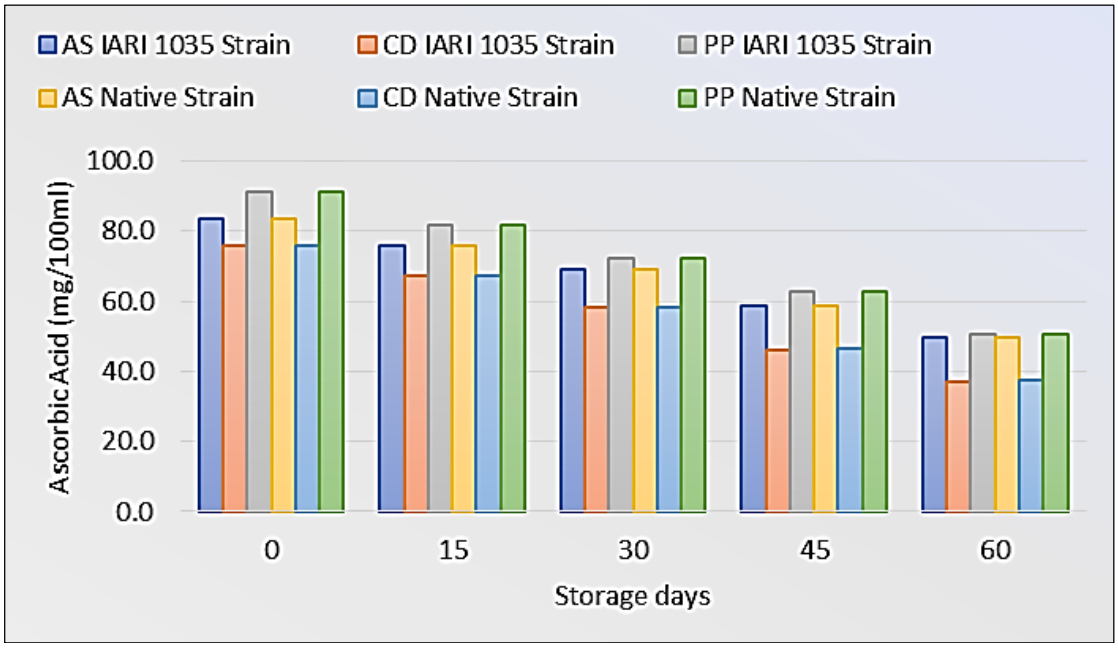

Fig 7: Variation in ascorbic acid content of guava wine with 12% inoculum concentration of *S. cerevisiae* IARI and Native strains

Table 1: Effect of inoculum concentrations, strains and storage period on ascorbic acid of guava wine samples

Ascorbic Acid (mg/100ml)																		
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	83.50	76.10	91.17	83.50	76.10	91.17	83.50	76.10	91.17	83.50	76.10	91.17	83.50	76.10	91.17	83.50	76.10	91.17
15	76.00	67.38	81.68	76.05	67.35	81.68	76.16	67.22	81.74	76.18	67.22	81.68	76.09	67.16	81.74	76.05	67.28	81.68
30	68.86	58.66	72.52	68.86	58.66	72.48	69.03	58.50	72.42	69.00	58.50	72.48	68.93	58.41	72.35	68.98	58.47	72.31
45	58.69	46.45	62.84	58.72	46.45	62.84	58.75	46.36	62.77	58.77	46.36	62.84	58.76	46.29	62.77	58.81	46.40	62.77
60	49.86	37.42	50.81	49.86	37.35	50.81	49.81	37.29	50.68	49.70	37.29	50.81	49.62	37.23	50.62	49.49	37.27	50.62

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

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 1.5 to 1.10 that the acidity of guava wine showed an increasing trend while the ascorbic acid of guava wine showed decreasing trend for all the treatments with storage period up to 60 days. The increase in acidity of guava wine

may be due to formation of organic acid by ascorbic acid degradation as well as progressive decrease in protein content. The study also revealed that acidity increased with increased in storage period irrespective of storage conditions.

Results present in Tables 1.1 and 1.2 revealed that the lowest value of acidity (1.27) was observed for the sample of Chittidar having 8% inoculum concentration with IARI strain and highest value of acidity (1.90) was observed for the sample of Punjab Pink having 8% inoculum concentration with IARI strain after 60 days. However, in general higher value of acidity was observed after 60 days irrespective of varieties, inoculum concentrations and strains. The lowest value of ascorbic acid (37.23) was observed for the sample of Chittidar having 12% inoculum concentration with S. cerevisiae 1035 and highest value of ascorbic acid (50.81) was observed for the sample of Punjab Pink having 4% inoculum concentration and both strain after 60 days. 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.

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