

Study of Fermentation Variables for the Preparation of Wine from Jamun Fruit

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The present investigation was conducted with the objective to explore the production of wine from *Jamun* fruit by manipulating the fermentation variables. During study three different wine yeasts strains viz. *Sachharomyces cerevisiae* 3304, 4787 and 3604 were first screened at 15°C and 25°C for their ability to produce wine using *Jamun* juice with respect to sugar utilization, alcohol production and yeast viable count, strain *S. cerevisiae* 4787 was selected on the basis of fermentation rate and final alcohol level in the wine. This strain was used for experiment carried out to investigate the effect of inoculum levels (2.5%, 5% and 7.5%) on the wine. Wine was chemically analyzed and observed that there was no significant effect of inoculum level. Considering the chemical composition of wines, yeast strain *S. cerevisiae* 4787, 7.5% inoculum level and fermentation temperature 25°C were optimum conditions for the preparation of wine. Wine thus prepared was evaluated chemically as well as organoleptically. It was observed that *Jamun* wine was quite acceptable and acceptability of wine was increased with storage.

Keywords: *Jamun* wine, inoculum level, *Sachharomyces cerevisiae*, fermentation.

Fruits are important source of minerals and vitamins and are important constituents of the diet. Fruits being perishable commodity cannot be transported to distant places for marketing. The surplus produce, therefore, needs to be consumed or processed immediately. Technologies have been developed for the utilization of most of the fruits for the production of Jam, Jelly, Squash, Syrup and dehydrated products. During processing of fruits, a considerable part of valuable nutrient such as vitamin is lost as a result of heat treatment. Fruit wine is a health drink resulting from complete or

partial alcoholic fermentation of fruit juices with the help of wine yeast (*Saccharomyces cerevisiae*) (Joshi and Attri, 1990). Being fruit based undistilled beverage wine retains all the minerals and vitamins. A Glucose Tolerance Factor (GTF) synthesized by yeast and released in fermentation medium is helpful in curing diabetes (Kraus, 2003). Wines are beverages with moderate alcoholic content (10-15%) and are consumed as an appetizer and stimulant in modern diet. Besides alcohol, wine contains vitamins, amino acids, esters, tartaric acid and phenolic compounds derived from the fruit juices and yeast. The phenolic compounds are effective in reducing thrombotic anthergenic process and also inhibit platelet aggregation (Simonetti et al. 1997). A large part of these phenolics in wine act as antioxidants and some of

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these are effective for lowering the lipid oxidation (Kanner et al. 1994) and has positive health effects against cancer and brain degenerative process (Budak and Guzel-Seydim, 2010). Proteins are also present in wines in low concentrations (15-230 mg/l), contributing little to their nutritive value and affect the clarity and stability of wines (Glampedaki et al. 2010)

Traditionally, Grapes have been used to produce different types of wines. However, other fruits such as apple, plum, apricot, peach, banana, and guava have also been utilized for the preparation of wine.

Jamun (*Syzygium cuminii* L. Skeels), a minor fruit crop, is gaining popularity among the consumers due to its high nutraceutical values (Das, 2009). *Jamun* belongs to the myrtaceae family, is cultivated for its colourful, edible fleshy fruits (Janick and Paull, 2008) and is a native of India, Burma (Myanmar), Sri Lanka. The other common names of *Jamun* are java plum, jambul, jambu, blackplum, and kalajam (Prasad and Kumar, 2010). *Jamun* fruits are available only for short duration in June-July. Its fruits are a rich source of minerals (Mn, Zn, Fe, Ca, Na etc.), sugars, tannins, anthocyanin and other phenolic compounds. *Jamun* contains 19.7% carbohydrates, 0.7% proteins, 0.02% calcium, 0.1% fat, 0.01% phosphorus, 0.4% mineral matter, 0.1% iron and 0.9% fiber (Rai et al. 2011). The ripe *Jamun* fruits are bright purple in color and delicious in taste having high nutritional and medicinal value. The berry is oblong, ovoid and shiny crimson black (rich in anthocyanins as antioxidant) when fully ripe (Chowdhury and Ray, 2007). The ripe fruits widely eaten and has combination of sweet mildly sour and astringent flavour. The presence of oxalic acid, gallic acid and certain alkaloids are responsible for the typical astringent taste. The refreshing and curative properties of *Jamun* make it one of the useful medicinal plants of India. *Jamun* fruits are good source of iron used as an effective medicine against diabetes, heart & liver trouble (Prasad and Kumar, 2010). Before the discovery of insulin, *Jamun* was useful in treatment of diabetes and as an integral part in the various alternative systems of medicine (Baliga et al, 2011). Attempts have been made to process *Jamun* into Jam, Jelly, Squash and Ready-to-Serve beverages. The fruit contains a biochemical called Jamboline which

is believed to check the pathological conversion of starch into sugar in case of increased level of glucose (Chowdhury and Ray, 2007).

In view of many medicinal and therapeutic properties of *Jamun* and seasonal availability, an attempt has been made in this study to prepare wine from its juice since wine has longer shelf life than fruit juices and retains all the nutrients.

MATERIALS AND METHODS

Jamun fruits and wine yeast

The fresh and ripened *Jamun* fruits were procured from local market of Hisar and used for preparation of wine. Pure cultures of *Sachharomyces cerevisiae* strain 3304 and 3604 were obtained from National Chemical Laboratory, Pune and strain 4787 was obtained from IMTECH, Chandigarh.

Extraction of juice

Ripe fruits were washed thoroughly with tap water containing 200ppm of potassium metabisulphite and the pulp was separated manually from the seeds. The pulp was then homogenized in the blender to obtain fine pulp and juice was extracted by hand pressing through muslin cloth.

Fermentation of juice

Cane sugar and tartaric acid were added into the juice (amelioration) to attain 20°Brix and pH 4.5 respectively. Then juice was supplemented with 0.2% ammonium sulphate to provide additional nitrogen for the growth of yeast. For the screening of yeast strains *Jamun* juice was inoculated with 5% starter culture of *S. cerevisiae* strains 3304, 4787 and 3604. Fermentation was carried out in flask containing 200ml juice at 15°C and 25°C. Samples were withdrawn at regular intervals for the chemical analysis. The observations were continued till fall in °Brix stayed. For further experiment strain *S. cerevisiae* 4787 was selected on the basis of fermentation rate and alcoholic content. To compare the effect of inoculum levels (2.5% and 7.5%) the *Jamun* juice was inoculated with starter culture strain *S. cerevisiae* 4787 and fermentation was carried out at 15°C and 25°C. The fall in °Brix was recorded regularly. The observations were continued till steady state values were obtained. The wine was then clarified by centrifugation at 5700 rpm for 10 minutes. After

that wine was bottled and pasteurized at 60°C for 20 minutes and stored at refrigerated temperature for two months. Considering the chemical composition of wines, yeast strain *S. cerevisiae* 4787, 7.5% inoculum level and fermentation temperature 25°C were optimum conditions for the preparation of wine. The *Jamun* wine obtained from this treatment was analysed for organoleptic acceptability.

Chemicals

The chemicals used for investigation were of analytical grade reagents (A.R.) obtained from Glaxo India Ltd., Bombay, E. Merck, India and Himedia.

Chemical Analysis

T.S.S was recorded by hand refractometer (0-32 scale) and pH was recorded with the help of pH meter. Acidity (%) and total phenols (mg/100ml) were analyzed as per the procedure suggested by AOAC (1990).

Sugars were estimated by the method developed by Lane and Eynon (1923). Alcohol contents in beverage were determined by methods of Caputi et al. (1968). Yeast viable count (CFU/ml) was determined by total plate count method.

Organoleptic Evaluation

100 point score card was used to record organoleptic quality of wine. The wine samples were evaluated organoleptically by 10 semi trained judges at 0, 30 and 60 day.

Statistical Analysis

The data obtained was subjected to analysis of variance (ANOVA) technique and analyzed according to factorial completely randomized designs (CRD). The critical difference

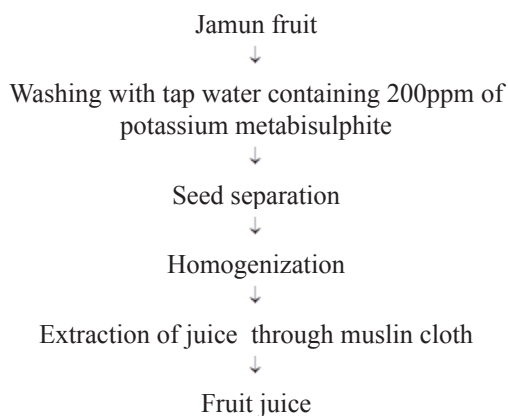


Fig. 1. Flow sheet for extraction of Jamun juice

value at 5% level was used for making comparison among different treatments.

RESULTS AND DISCUSSION

During fermentation of fruit juices for the production of wine, utilization of sugars by the yeasts results in production of alcohol and fall in °Brix of the medium. Simultaneously, other chemical changes take place, production of organic acids results in fall in pH and increase in acidity. The viable yeast count also increases and at a certain stage it reaches a plateau and then decreases. These changes may vary depending on fruit juice composition, stages of fermentation, pH and temperature of incubation. All these factors are characteristics of yeast strain and composition of fermentation medium. In the present work, efforts have been made to monitor these variations and their effect on wine quality and acceptability has been recorded.

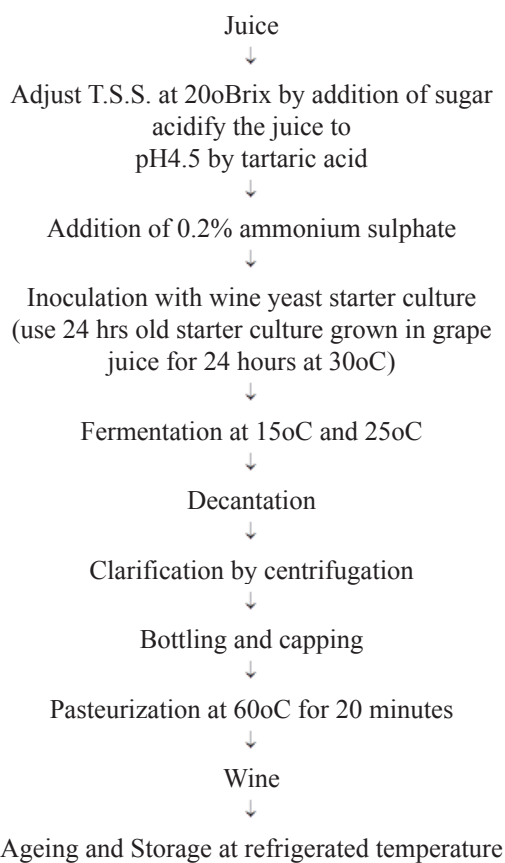


Fig. 2. Flow sheet for preparation of wine

Screening of yeast strains *S. cerevisiae* 3304, 4787 and 3604 for wine production from Jamun juice

Strains : 3304 (I) 4787 (II) 3604 (III)

Inoculum level : 5%

Three different wine yeasts strains viz *S. cerevisiae* 3304, 4787 and 3604 were first screened for their ability to produce wine using *Jamun* juice with respect to sugar utilization, alcohol production and yeast viable count.

T.S.S.

Results pertaining to progress of fermentation of *Jamun* juice are shown in table 1 it is evident from the data that fermentation was faster during second and fourth day of fermentation. The reason probably could be the high nutrient level and low alcohol content in fermentation medium. During this period decline in °Brix with proportionate increase in alcohol content was

observed. Similar observations have been reported by Chavan (2008); Joshi et al. (1997). However, there was faster decrease in °Brix in case of strain I and lesser in case of strain III. Effect of temperature was also found to be significant. The fall in °Brix was faster at higher temperature i.e. at 25°C as compared to 15°C.

pH

Table 2 reveals that there was significant effect of temperature and strains on the pH of the fermentation medium during fermentation. In case of strain II the pH decrease from 4.5 to 3.5 which was lower as compared to other two strains (4.5-3.9). The possible reason could be production of organic acids as a result of metabolism of sugar by yeast during fermentation. This observation is in accordance with those of Chowdhury and Ray (2007); Shukla et al. (1991).

Table 1. Effect of different strains on the brix of *Jamun* juice at different temperature during fermentation

Strain → Temperature →	I			II			III			F.T × T		
	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	0	20	20	20	20	20	20	20	20	20	20	20
	2	16	15.9	15.9	16.4	16.2	16.3	19	18.8	18.9	17.1	16.9
	4	7	5.6	6.3	8.0	5.6	6.8	14.6	14	14.3	9.8	8.4
	6	6	5	5.5	6	5	5.5	10	7.4	8.7	7.3	5.8
	8	6	5	5.5	6	5	5.5	7	6	6.5	6.3	5.3
	Mean	11	10.3	10.7	11.2	10.3	10.8	14.1	13.2	13.7	12.1	11.3

CD at 5%

Fermentation time (F.T) : 0.09 Temperature (T) : 0.06 Strain (S) : 0.07

S × T : 0.09 F.T × T : 0.12 F.T × S : 0.15

F.T × S × T : 0.22

Table 2. Effect of different strains on the pH of *Jamun* juice at different temperature during fermentation

Strain → Temperature →	I			II			III			F.T × T		
	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
	2	4.4	4.3	4.3	4.3	4.4	4.3	4.4	4.4	4.4	4.4	4.4
	4	4.3	4.2	4.2	4.2	3.7	3.9	4.3	4.3	4.3	4.2	4.1
	6	4.2	4.2	4.2	4.2	3.5	3.8	4.2	4.0	4.1	4.2	3.9
	8	3.9	3.9	3.9	3.8	3.3	3.5	4.0	3.9	3.9	3.9	3.7
	Mean	4.3	4.2	4.2	4.2	3.9	4.0	4.3	4.2	4.3	4.2	4.1

C.D at 5%

Fermentation time (F.T) : 0.06 Strain (S) : 0.04 Temperature : 0.04

F.T × S : 0.09 F.T × T : 0.08 S × T : 0.06

F.T × S × T : 0.14

Alcohol (%)

Increased in alcohol level during fermentation is presented in table 3. It was observed that the strain II produced more alcohol (11.3%) as compared to strain I (10.0%) and strain III (9%). Although in normal cases it should have

been around 12%-14%. The probable reason of observed result could be high level of residual (non-fermentable) sugar (~6%) in the final product. This is expected in fruit juice having relatively lower levels reducing sugars. Alcohol production was found to be faster at 25°C as compared to 15°C

Table 3. Effect of different strains on the alcohol (%) of Jamun wine at different temperature during fermentation

Strain → Temperature →	I			II			III			F.T × T			
	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean	
Fermentation time (Days)	2	2.6	3.0	2.8	2.7	3.8	3.2	1.6	1.6	1.6	2.3	2.8	2.6
	4	4.0	5.1	4.6	4.0	6.0	5.0	3.0	3.0	3.0	3.6	4.7	4.2
	6	9.4	10.0	9.7	10.1	11.3	10.7	7.1	7.0	7.0	8.9	9.4	9.2
	8	10.0	10.0	10.0	11.0	11.3	11.1	9.0	9.0	9.0	10.0	10.1	10.1
	Mean	6.52	7.05	6.79	6.95	8.11	7.53	5.17	5.17	5.17	6.22	6.78	6.50

C.D at 5%

Fermentation time (F.T) : 0.12 Strain (S) : 0.10 Temperature (T) : 0.08
 F.T × S : 0.20 F.T × T : 0.17 S × T : 0.14
 F.T × S × T : 0.29

Table 4. Effect of different treatments on viable yeast count (CFU/ml) during fermentation of *Jamun* wine

Strain → Temperature →		I		II		III	
		15°C	25°C	15°C	25°C	15°C	25°C
Fermentation time (Days)	0	8.0×10 ⁶	8.5×10 ⁶	9.0×10 ⁶	1.0×10 ⁷	1.1×10 ⁷	1.8×10 ⁷
	2	1.8×10 ⁸	2.5×10 ⁸	1.3×10 ⁸	1.5×10 ⁸	1.2×10 ⁸	1.3×10 ⁸
	4	2.6×10 ⁸	3.5×10 ⁸	2.1×10 ⁸	2.5×10 ⁸	1.9×10 ⁸	2.0×10 ⁸
	6	7.8×10 ⁷	1.0×10 ⁸	8.8×10 ⁷	1.4×10 ⁸	1.3×10 ⁸	9.1×10 ⁷
	8	2.5×10 ⁷	4.0×10 ⁶	4.1×10 ⁷	1.4×10 ⁷	3.0×10 ⁷	2.5×10 ⁷

Table 5. Effect of inoculum level on the brix of Jamun juice at different temperature during fermentation

Inoculum Level → Temperature →	2.5			7.5			F.T × T		
	15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	0	20	20	20	20	20	20	20	20
	2	14	12	13	14	12	13	14	13
	4	10	8	9	8	7	7.9	9	8
	6	6	6	6	6	6	6	6	6
	8	6	6	6	6	6	6	6	6
	Mean	11.2	10.4	10.8	10.9	10.2	10.6	11.0	10.3

CD at 5%

Fermentation time (F.T) : 0.11 Inoculum level (I.L) : 0.07 Temperature (T) : 0.07
 F.T × I.L : 0.16 F.T × T : 0.16 I.L × T : N.S
 F.T × I.L × T : N.S

Table 6. Effect of inoculum level on pH of Jamun juice at different temperature during fermentation

Inoculum Level → Temperature →		2.5			7.5			F.T × T		
		15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	0	4.4	4.5	4.5	4.4	4.5	4.5	4.5	4.5	4.5
	2	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
	4	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3	4.3
	6	4.0	3.8	3.9	4.2	3.9	4.0	4.1	3.8	3.9
	8	4.0	3.8	3.9	4.2	3.9	4.0	4.1	3.8	3.9
	Mean	4.3	4.2	4.2	4.3	4.2	4.3	4.2	4.3	4.2

CD at 5%

Fermentation time (F.T) : 0.01 Inoculum level (I.L) : 0.01 Temperature (T) : 0.01

F.T × I.L : 0.01 F.T × T : 0.01 I.L × T : 0.01

F.T × I.L × T : 0.01

Table 7. Effect of Inoculum level on total sugar (g/l) of Jamun juice at different temperature during fermentation

Inoculum Level → Temperature →		2.5			7.5			F.T × T		
		15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	0	178	180	179	182	172	177	180	176	178
	2	110	85	97	92	81	87	101	83	92
	4	65	50	57	61	60	61	63	55	58
	6	58	39	49	47	41	44	53	40	47
	8	43	35	39	40	36	38	42	36	39
	Mean	90.9	78.0	84.4	84.7	78.2	81.4	87.8	78.1	82.9

CD at 5%

Fermentation time (F.T) : 2.04 Inoculum level (I.L) : 1.26 Temperature (T) : 1.26

F.T × I.L : N.S F.T × T : 2.90 I.L × T : 1.83

F.T × I.L × T : 4.10

Table 8. Effect of Inoculum level on alcohol content (%) of Jamun wine at different temperature during fermentation

Inoculum Level → Temperature →		2.5			7.5			F.T × T		
		15°C	25°C	Mean	15°C	25°C	Mean	15°C	25°C	Mean
Fermentation time (Days)	2	1.6	2.2	1.9	1.6	3.0	2.3	1.6	2.6	2.1
	4	2.3	4.5	3.4	2.0	3.5	2.8	2.2	4.0	3.1
	6	7.0	7.5	7.2	7.0	7.8	7.4	7.0	7.6	7.3
	8	9.0	9.0	9.0	8.0	9.0	8.5	8.5	9.0	8.8
	Mean	4.9	5.8	5.4	4.6	5.8	5.3	4.8	5.8	5.3

CD at 5%

Fermentation time (F.T) : 0.20 Inoculum level (I.L) : 0.14 Temperature (T) : 0.14

F.T × I.L : 0.28 F.T × T : 0.28 I.L × T : 0.20

F.T × I.L × T : 0.39

Table 9. Chemical composition of *Jamun* wine

Parameters	Mean*±S.D <i>Jamun</i> wine
T.S.S(°Brix)	6
pH	4.2±0.03
Acidity (%)	0.70±0.01
Total sugar(g/l)	42±0.02
Reducing sugar (g/l)	35±0.02
Phenols (mg/100ml)	60±0.04
Anthocyanins (mg/100ml)	60±4.50
Alcohol (%)	8 ±0.02

*The values are mean of three replications

in the initial stages. But at the end of fermentation there was no significant effect of temperature on alcohol content of the wine. Interaction of strain, temperature and fermentation time were found to be significant. Chowdhury and Ray (2007) observed 6% alcohol in *Jamun* wine. This was probably due to low T.S.S (16.5°Brix) in *Jamun* juice.

Yeast viable count (CFU/ml)

Table 4 reveals that higher temperature (25°C) favoured yeast multiplication in the initial stages of fermentation but at the end of fermentation (8th day) viable cell count decreased probably due to inhibitory action of alcohol. Similar observations

Table 10. Effect of different treatments on organoleptic quality of *Jamun* wine

Storage time (Days) ⁻	Color/appearance (20)	Taste (20)	Body (20)	Aroma (20)	Astringency (20)	Overall acceptability
0	16.8 ±0.9	12.9 ±0.3	16.1 ±0.3	14.5 ±0.5	8.7 ±0.4	69.0 ±1.4
30	18.4 ±0.2	14.9 ±0.4	17.4 ±0.4	14.5 ±0.3	8.9± 0.2	74.1 ±0.8
60	17.7 ±0.4	15.8± 0.2	18.0 ±0.4	16.0 ±0.3	14.1 ±0.7	81.6 ±1.2
CD (< 0.05%)	N.S.	0.9	1.2	1.3	1.5	3.8

have also been reported by Charoenchai et al. (1998).

Wine yeast strain 4787 was selected as best strain on the basis of fermentation rate and final alcoholic content of wine. This strain was used for experiment carried out to investigate the effect of inoculum levels (2.5%, 5% and 7.5%) on the wine.

Effect of inoculum level

T.S.S and pH

It was observed that fermentation was faster in fermentation medium inoculated with 7.5% inoculum level as compared to 2.5% (table 5). This was due to higher yeast count. However, at the end of fermentation inoculum level has no significant effect on °Brix. It could be due to production of more alcohol earlier in case of 7.5% as compared to 2.5% inoculum level.

The inoculum level had no significant effect on the final pH of the medium (table 6).

Total sugars

Table 7 shows the decrease in total sugar content of fermentation medium during fermentation. The residual sugar was in range of 35-43 g/l in wine prepared from juice inoculated

with 2.5% inoculum level as compared to juice with 7.5% inoculum level (36-40 g/l). This may be due to the higher yeast count in case of 7.5% inoculum level which utilizes more sugar as compared to lower yeast count in case of 2.5% inoculum level.

Alcohol (%)

Gradual increase in alcohol content was observed during fermentation (table 8). Alcohol content was lower in wine prepared with 7.5% inoculum level (8.5%) as compared to 2.5% and 5% inoculum level (9% and 11% respectively). This may be due to the decrease in yeast activity due to earlier production of alcohol in case of 7.5 % inoculum level. The higher alcohol level in wine with 5% inoculum level probably could be due to optimum utilization of sugar as compared to sugar utilization in case of 2.5% inoculum level. The interaction of inoculum level, fermentation time and temperature was found to be significant. The above results are in agreement with reports of Borate et al. (2008).

It was observed that 2.5% inoculum level was sufficient to produce alcohol. But to avoid the contamination in the juice was inoculated with 7.5% inoculum level. At such high inoculum

level yeast growth dominates the growth of other contaminants or prevents the growth of other microbes.

On the basis of chemical analysis it was found that strain *S. cerevisiae* 4787, 7.5% inoculum level and 25°C fermentation temperature were best treatments for preparation of wine. The wine was evaluated chemically (table 9) as well as organoleptically (at 0, 30 and 60 day) (table 10).

It was observed that *Jamun* wine was acceptable to all panellists in spite of its higher astringency which is due to the presence of phenolic compounds. It was also observed that on prolonged storage the acceptability of wine increased due to reduction of phenolic compounds and yeast odour on storage. Shukla et al. (1991) reported that *Jamun* wine got 83 organoleptic score on 100 point score card. However, Borate et al. (2008) studied the wine prepared from *Jamun* juice with treatments, 24°Brix TSS, 7.5% inoculum level and 15 fermentation days and reported that wine got 17.93 overall acceptability score out of 20.

CONCLUSIONS

Based on our results, it has been concluded that out of three strains tested strain *S. cerevisiae* 4787 is suitable for fermentation of *Jamun* juice as the final alcohol percentage was higher as compared to other strains at the end of eight days of fermentation. There was no significant effect of inoculum level on the fermentation rate and quick fermentation was found at 25°C. *Jamun* wine was found to be quite acceptable and observed that acceptability increased with storage.

However, considering the therapeutic and medicinal value of *Jamun* for diabetic persons and short shelf life of *Jamun*, it can be used for preparation of wine as all the nutrients of fruits are retained in wine after fermentation of fruit juice. Finally it can be concluded that *Jamun* fruit can be utilized for wine making process to provide a new value product from *Jamun*.

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