Efficacy of Yeast Strains on Fruit Wine Fermentation

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Abstract:

The present study was carried out during 2016-2018 with the aim of developing quality wine from jackfruit and identifying efficient yeast strains for production of fruit wine. Feasibility of making wines from the fruits was investigated through bio-chemical, sensory and microbial examination of the developed wines. We used in this study 4 local isolates (LI-1, LI-2, LI-3 and LI-4), *and 1 strain from National Chemical Laboratory, Pune (NCIM 3189), and 2 strains from the Department of Agriculture Biotechnology (ADJ-1 and ADJ-2).*

Results showed that a yeast strain NCIM 3189 was suitable for production of wines from jackfruit. Where on the basis of organoleptic qualities, NCIM-3189 wine gave the highest score for overall acceptability, while the lowest score was obtained by ADJ-2wine. In terms of color and clarity also NCIM-3189 wines fared very good; while ADJ-2 wine obtained the lowest score. Reverse bitterness score was highest in LI-1 wine and acidity in NCIM-3189wine. Both ADJ-1and ADJ-2wines got the lowest scores for acidity. For taste and flavor, the highest score was obtained in NCIM-3189 wine and the lowest was obtained in ADJ-2 wine. The clarity was highest in NCIM-3189 wine and lowest in ADJ-2wine.Sensory evaluation categorized the wines into three distinct groups, the first group comprising LI-1, LI-3 and NCIM 3189 wines, of which, mean values were above the global mean, the second group with LI-2 and LI-4 wine the means of which were at par with the global mean, and the third group comprising ADJ-1 and ADJ-2 the means of which were below the global mean. Within the first group, NCIM 3189 topped with a mean of 7.07 which clearly distinguished NCIM 3189 as the mostly liked wine by the panelists.

Keywords: *Wine, yeast strain, bio chemical examination, sensory examination and microbial examination.*

I. INTRODUCTION

Wine making is practised from the ancient times which is now a commercially prosperous biotechnological ventures [1]. Since ancient times, wine has been an integral part of the diet, particularly for people in Mediterranean countries. Several studies have established health benefits associated with consumption of wine. Moderate consumption wine, especially red may help people live longer, protect against certain cancers, improve mental health, and enhance heart health, and exert positive effects on lung function, antioxidant capacity, lipid profile and the coagulation system, that reduces the risk of cardiovascular disease, overall mortality and other diseases [2].

Red wine consumption cuts the risk of lung cancer, moderate drinking increases the bone mass in elderly women and also reduces the risk of type-2 diabetes [3]. Grape red wine contains resveratrol which protects the consumer from coronary heart diseases in addition to having anti-inflammatory, antioxidant and anti-tumour effects [4].

The fermentation process leading to alcohol production largely depends on the ability of yeast strains to convert sugars into alcohol, esters and other volatile and non-volatile compounds[5]. Due to differences in fruit composition, the yeast strains used for fermentation must adapt to different environments like sugar composition and concentration of organic acids [6].

Production of wine from fruits other than grapes has increased in the recent years. Any fruit which has good proportion of sugar can be used for wine making. Wines are mostly named after the fruit from which it has been prepared. Various researches have established the use of other fruits as the potential raw material for wine making [7].

Jackfruit (*Artocarpus heterophyllus* L.) is indigenous to India and is widely grown in Bangladesh, Burma, Sri Lanka, Malaysia, Indonesia, Philippines, Brazil and other tropical countries [8]. The fruit juice should contain at least 14 per cent (w/w) of sugar to be converted into alcohol. If the sugar content is less than 14 % w/w, some amount of sugar should be added to increase the sugar level. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production. The initial sugar concentrations, fermentation temperatures, SO_2 concentrations added during fermentation phase and specific yeast strains are key factors in determining successful fermentative processes of fruit wine [9]. At the moment, most of the wine production processes rely on *Saccharomyces cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck

fermentations and prevent microbial contaminations [10].

Selection of proper yeast strains is one of the major factors in production of good quality wine [11]. 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 selection process of yeast strains depends on their oenological characteristics, such as fermentative rate, tolerance to ethanol and SO2, flocculent characteristics, the presence of killer factors, acetic acid production, H2S, malic acid metabolism, higher alcohol production, alcohol yield, glycerol production, and extra cellular enzyme production [12].

Natural wine fermentation combines the activities of several yeasts species, which grow sequentially throughout the fermentation process. The process is initiated by various species of *Candida*, *Debaryomyces*, *Hanseniaspora*, *Pichia*, *Kloeckera*, *Metschnikowia*, *Schizosaccharomyces*, *Torulospora*, and *Zygosaccharomyces*, which naturally exist on the grape surface. Yeast growth is generally limited to the first 2 or 3 days of fermentation due to osmotic pressure caused by the glucose added. Subsequently, the most strongly fermenting and more ethanol tolerant species of Saccharomyces dominates the fermentation [13]. However, modern winemaking is founded on the use of selected commercial *S. cerevisiae* strains for their reliable properties, which contribute to the quality of the resulting product.

Considering the above aspects, the present investigation was formulated to Identification of efficient yeast strains for production of fruit wine and to assessment of quality of the developed wine across storage.

II. MATERIALS AND METHODS

The experiment was carried out during 2016-18 at the Quality Assurance and PHT Laboratory of the Department of Horticulture and the Microbiology Laboratory of the Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat. Locally available fresh jackfruitwas evaluated for their suitability for production of wine.

A. Raw Materials Used

Jackfruit pulp (*Artocarpus heterophyllus* L.) was taken as the ideal food system for screening of the yeast strains and the ripe fruits were collected from the Experimental Farm of the Department of Horticulture for use in the experimentation.

B. Extraction of Juice

Jackfruit was cut and the ripe bulbs were taken out and the seeds were removed. Juice was extracted with the help of a screw type juice extractor.

C. Evaluation of Raw Materials

a) Determination of titratable acidity:

The titratable acidity of the samples was estimated by volumetric method [14]. The samples were extracted and filtered. Five mLaliquot was taken in a 50mL volumetric flask and the volume was made up with distilled water. 5 mL of this was titrated against 0.1N NaOH using phenolphthalein indicator. In case of mulberry samples, a pH meter was used to determine the end point in place of the indicator. NaOH was added drop wise, and respective volume of alkali used and the pH were noted. The process was continued till the pH of the sample reached 9.0. Through interpolation on a graph paper, the respective volume of alkali consumed was noted against pH 8.2 (the pH at which phenolphthalein changes colour). Acidity, in terms of lactic acid, was calculated using the following formula:

$$\% \text{ acidity as lactic acid} = \frac{Titrevalex Normality x Equivalentweig\ htoftheacid x Volumemadeup}{Weightofthesampletakenforestimation x Weig\ htofsamplex\ 1000} x\ 100$$

b) Total soluble solids

The TSS was read using a digital hand refractometer (ATACO, Tokyo) and expressed in °Brix.

c) Determination of total phenols content:

The total phenol content of wines was determined spectrophotometrically as per the Folin-Ciocalteu method as described by [15]. One mL of wine was mixed with 1.8mL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and incubated at room temperature for 5min followed by the addition of 1.2mL of sodium carbonate (15%w/v). The mixture was incubated for 90min at room temperature and the absorbance was recorded at 650nm in an UV-visible spectrophotometer (Varian Cary 50 spectrophotometer). The total phenols content of the samples was compared with the standard curve of catechol and expressed as g 100 $mL^{-1}$of catechol equivalents.

d) Estimation of carotenoid content (vit A)

The total carotenoid was determined according to [16]. Five g sample with 3 g celite powder were mixed and ground with 50 mL cold acetone and filtered through Whatman no.4 filter paper. Forty mL petroleum ether was added to the filtrate in a 500 mL separating funnel. The solution was washed 3-4 times with distilled water to discard the lower aqueous phase without discarding the upper phase. The upper phase was collected in 50 ml volumetric flask and 15 g of anhydrous sodium sulphate was added to remove the residual water. The solution was again filtered and volume was made up with petroleum ether. The absorbance was recorded at 450 nm in a UV-VIS spectrophotometer and total carotenoid content (µg) was calculated with the following relation:

$$\text{Total carotenoid } (\mu g g^{-1}) = \frac{absrbance \times Volume\ (mL) \times 10^4}{absorbance\ \ coeffici\ ent \times Weig\ htofsample\ \ (g)}$$

e) Estimation of total sugars:

The sugar content of the samples was estimated by Anthrone method [17]. Hundred mg of the samples was taken into a tube and hydrolyzed by keeping it in a boiling water bath for 3h with 5mL of 2.5N HCl and cooled to room temperature. It was then neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100 mL and centrifuged. The supernatant was collected and, 0.5 and 1mL aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. All the volumes of the tubes were made up to 1ml including the sample tubes by adding distilled water. Then, 4mL of Anthrone reagent was added and heated for 8min in a boiling water bath. The samples were cooled rapidly and read at 630nm in spectrophotometer. A standard graph was drawn by plotting concentration of the standard on the X-axis *vs.* absorbance on the Y-axis. From the graph, the amount of sugars present in the sample tube was calculated.

D. Preparation of Must

The juice was extracted from the fruits and equal volume of water was added to dilute the juice [4], [15]. The must was prepared by raising the TSS to 20°Brix [18] by extraneous addition of sugar.

E. Preparation of Active Yeast Culture

Four lots of jackfruit pulp were allowed for natural fermentation inside the laboratory and four isolates (LI-1, 2, 3 and 4) were obtained and used subsequently in experimentation (Table 1).

The pure culture was maintained on agar slant by monthly transfer holding at 4 °C between each transfer and sub-culturing 3 to 4 times in MGYP nutrient broth having 0.3% malt extract, 1% glucose, 0.3% yeast extract, 0.5% peptone, 100mL distilled water with the pH adjusted to 6.4-6.8.

Table (1): The strains/isolates used during experimentation

Strain/Isolate No.	Strain/isolates
LI-1	Isolate from natural fermentation of jackfruit pulp
LI-2	Isolate from natural fermentation of jackfruit pulp
LI-3	Isolate from natural fermentation of jackfruit pulp
LI-4	Isolate from natural fermentation of jackfruit pulp
ADJ-1	*Saccharomyces cerevisiae* ADJ-1(NCBI accession no KX904345) collected from Dept. of Agril. Biotechnology, AAU, Jorhat
ADJ-2	*Wickerhamomyces anomalus* ADJ-2 (NCBI accession no KX904346) collected from Dept. of Agril. Biotechnology, AAU, Jorhat
NCIM 3189	*Saccharomyces cerevisiae* NCIM 3189 collected from National Chemical Laboratory, Pune

F. Addition of Active Yeast Culture and Fermentation

The active yeast was added to the prepared must at the rate of 5% (since 5% inoculum obtained highest alcohol percent when inoculated in sucrose solution). Fermentation was carried out in bottles with their lids connected with a pipe for the passage of CO_2, one end of the pipe inside the bottle and the outer end stoppered with a 0.2µ PTFE membrane filter. Before starting the fermentation, the pH of the must was adjusted at 4.5±0.5 by either adding 1M citric acid or 1M sodium bicarbonate. The fermentation was stopped on 12days. The wine was transferred to another flask having aeration and aqueous solution of bentonite ($10gL^{-1}$)was added to facilitate the sedimentation of non-fermentable solids. The mixture was then homogenized and incubated at 8°Cfor 48h for sedimentation of flocculent material [19], [20].

The wines were then was pasteurized at 60^oC for 30min [21]. The wines were stored at room temperature (25±2 °C). All assays were carried out in triplicate. The method of preparation is given in (Figure 1).

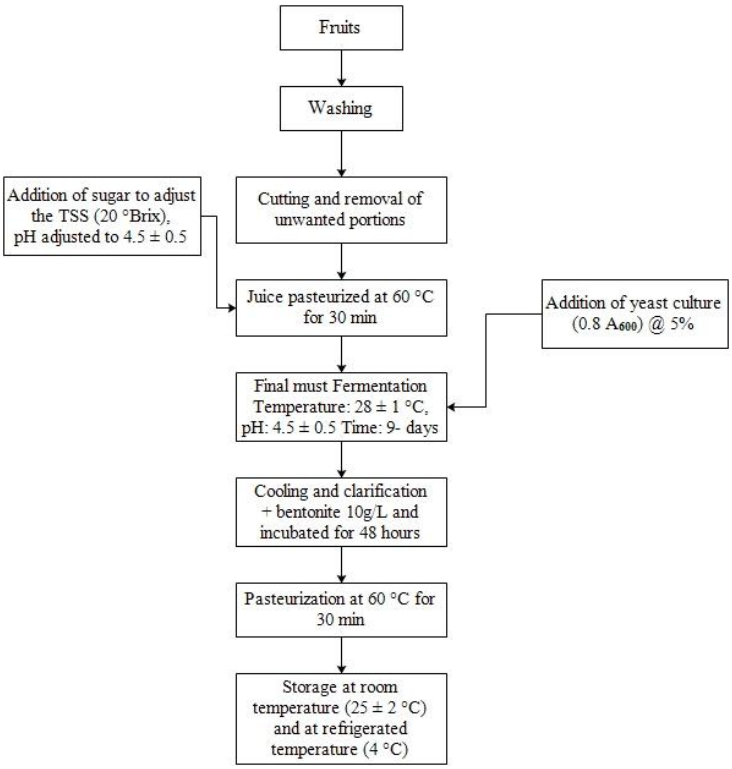

Fig. (1): Flow chart of wine preparation

G. Qualitative Analysis of Wine

a) Total soluble solid (TSS) and Titratable acidity:

Determined as per the procedure described above.

b) CIE Lab colour parameters

CIE L*a*b* values of prepared wine samples were determined by HunterLabColourQuest XE Colorimeter (Hunter Associates Laboratory, Inc., Virginia, USA), and H* and C* values were calculated by the following equations:

Hue (H*) = Tan^{-1} b/a

Chroma (C*) = $\sqrt{a^2 + b^2}$

c) Alcohol content (% v/v)

Alcohol content (% v/v) of the wines was measured by using gas liquid chromatography as per [22].

d) Preparation of standard solutions: Standard were prepared by taking 1 mL of 99.5 % ethanol (Sigma-Aldrich, Cat. No. 900642) by adding 9 mL HPLC grade water to it.

e) GC-FID analysis: The samples were analysed using GC-FID with manual injection along with a blankconsisting of HPLC grade and standard solutions. The standard solutions were injected first followed by the blank. Every standard solution and sample was injected in triplicates. The inlet was set at 225 °C with split injection and a split ratio of 50:1. The injection volume was 1 µL. The column used was a Phenomenex ZB-FFAP GC-column of 30 m x 320 µmx0.25 µm nominal. The oven was set at a program methathadaninitial temperature of 45° C for 2 min that increased to 245°C, 45°C min^{-1}. It was held at 245°C for 1 min. The flame ionisation detector (FID) temperature was set at 285°C with a flow of 30 mL min^{-1} H_2. The flow rate of O_2 was set at 300 mL min^{-1}. The injection syringe was rinsed with HPLC grade water between every injection.

f) Viscosity

The viscosity of the juice samples were measured by using Fungi labViscobasic Plus viscometer. The value was measured using L1 spindle at 100 rpm and expressed at centipoises (cP).

g) Estimation of total carbohydrates, carotenoid content and total phenols content:

Determined as per the procedure described above.

h) Microbial growth and yeast growth kinetics count determination

Serial dilutions of wines in sterile peptone water was done up to 10^{-6} dilution for viable

cell counts. Aliquot of 0.1mL of the wine samples was inoculated on plate count agar, potato dextrose agar, eosin methylene blue (EMB) agar and MRS agar by spread plate method. Samples were seeded in triplicate. The plates were incubated at 30±2°C for 48h to check any microbial contaminations.

i) Sensory evaluation

The prepared wines were evaluated by 10 panellistson a 9point rating scale. The sensory scores were evaluated by product characterization test statistically by XLSTAT ver. 2016.02.27444

j) Statistical analysis

The experiment was carried out using Completely Randomized Block design replicated three times.

III. RESULTS AND DISCUSSION:

The results obtained from the present investigation are presented below:

A. Evaluation of Raw Materials

The data for TSS, acidity, carbohydrates, phenols, carotenoids and alcohol content in the raw materials are presented in (Table 2).

Table (2): Physico-chemical composition of ripe jackfruit

Component	Content
TSS(°Brix)	14.66±0.57
Acidity(%)	0.23±0.05
Carotenoid(µg100g^{-1})	179.49±0.01
Total sugars (g100g^{-1})	14.23±0.06
Phenols(mg100g^{-1})	3.20±0.44

The pulp of ripe jackfruit pulp contained TSS (°Brix) 14.66 ± 0.57, acidity (%) 0.23 ± 0.05, total sugars (g 100g^{-1}) 14.23 ± 0.06, phenols (mg100g^{-1}) 3.20 ± 0.44, and carotenoid (µg 100g^{-1}) 179.49 ± 0.01 (Table 2)

B. Biochemical Parameters of the Developed Wines

Data of alcohol content of seven different strains are presented in Table (3). The highest alcohol content was found in NCIM-3189(9.66%), ADJ-2 (9.56%) and ADJ-1 (9.50%) which were statistically *at par*.; while LI-4 wine obtained the lowest alcohol content (5.1%). TSS content of the wines was highest in LI-4 wine (2.02 °Brix) and the lowest was observed in NCIM 3189 wine (0.15 °Brix). The developed wines obtained alcohol content in the range of 5.1 to 9.66 per cent (v/v). Similar range of results were also reported earlier [23], [15] in jackfruit wines. Empirical studies have shown that the alcohol yield depends upon initial sugar concentration [24], temperature of fermentation [25], yeast strain and acidity [26], and other factors. The highest total sugar content was observed in LI-4 (3.65g 100mL^{-1}) LI-3 (0.63 g 100mL^{-1}) and LI-2 (0.57 g 100mL^{-1}) which were *at par*. The lowest sugar content was recorded in NCIM-3189 (0.11g 100mL^{-1}) and ADJ-2 (3.19g 100mL^{-1}) wines. Viscosity of the wines was highest in NCIM-3189 (4.20cP) with the lowest in LI-3 (2.46 cP). The acidity was maximum in NCIM3189 (0.42%) andLI-2(0.41%) wines, which were statistically *at par* and the lowest content was observed in ADJ-1 (0.13%) and ADJ-2 (0.14%). Wine phenolics were highest in NCIM 3189 (0.97g 100mL^{-1}) andADJ-2 (0.96g 100mL^{-1}) wine and, the lowest inLI-4 (0.56g100mL^{-1}). The lowest phenol content was recorded in ADJ-1 (7.19 mg100mL^{-1}). Total carotenoids were highest in NCIM 3189 (12.46µg 100mL^{-1}) and the lowest in ADJ-1 (11.83µg 100mL^{-1}) wine (Table 3).

The quality and variation of phenolics in wines depend on a wide range of factors, including cultural practices of the fruit trees, local climatic conditions, fermentation technologies and storage [27], [28].

Table (3): Biochemical composition of the developed wines

Wines	Alcohol content (%v/v)	TSS (°Brix)	Total sugar (g 100 mL^{-1})	Viscosity (cP)	Acidity %	Total phenol (g100mL^{-1})	Total carotenoid (µg 100mL^{-1})
LI-1	6.83	1.07	3.45	3.93	0.34	0.63	12.27
LI-2	7.46	1.03	3.57	3.26	0.41	0.82	12.12
LI-3	7.76	1.05	3.63	2.46	0.26	0.78	12.30
LI-4	5.10	2.02	3.65	3.16	0.21	0.56	12.10

ADJ-1	9.50	0.31	3.19	3.36	0.13	0.69	11.83
ADJ-2	9.56	0.30	3.23	2.93	0.14	0.96	12.14
NCIM 3189	9.66	0.15	3.11	4.20	0.42	0.97	12.46
S Ed(±)	0.09	0.07	0.05	0.08	0.02	0.05	0.05
CD=(0.05)	0.21	0.16	0.11	0.19	0.06	0.13	0.17

Data on the colour parameters of the wine are furnished in Table (4) LI-1 wineobtained the highest L*value of 61.81 followed by LI-3 wine with 53.58. ADJ-2 wine recorded the lowest L*value of 14.77. Redness was maximum in LI-1 wine as evidenced by the highest a* value (8.76) and the lowest in ADJ-1 wine (1.84). LI-1 wine recorded the highest b* value (42.41) which indicated yellowness, while ADJ-2 wine showed the lowest b* value of 11.21. The highest H* value was found in LI-3(85.13), ADJ-1 (84.17) and NCIM 3189 (83.53) wines which were statistically *at par*.The lowest*at par* values of H* were recorded in LI-1 (78.32), LI-2 (78.88), LI-4 (79.24) and ADJ-2 (79.27) wines. LI-1 wine showed the highest chroma with C* value of 43.31, while the lowest C* value was observed in ADJ-2 wine (11.54) (Table 4).

This indicated that, with increase in alcohol levels, luminosity L* decreased giving a lighter colour to the wine along with low redness a* values with lower degradation products and, higher yellowness b* values contributing yellowish tinge to the higher alcohol wines. The hue H* angles also ranged from 78.32 to 85.13 remaining in the first quadrant and tilting towards perfect yellow (90). The chroma C* ranged from 11.54 to 43.31 indicating saturation of the colour in the wines.

Table (4): CIE L*a*b* values of the developed wines

Wines	L*	a*	b*	H*	C*
LI-1	61.81	8.76	42.41	78.32	43.31
LI-2	34.32	5.31	27.06	78.88	27.57
LI-3	53.58	2.95	34.67	85.13	34.79
LI-4	30.23	3.47	18.29	79.24	18.62
ADJ-1	24.48	1.84	18.04	84.17	18.13
ADJ-2	14.77	2.72	11.21	79.27	11.54
NCIM3189	45.63	3.44	30.35	83.53	30.54
S Ed(±)	0.63	0.31	0.50	1.03	0.54
CD=(0.05)	1.34	0.64	0.87	2.41	1.17

C). Sensory Evaluation of the Developed Wines

All the developed wines obtained good ratings in sensory evaluation as shown in Table (5) NCIM-3189 wine was rated with the highest score (7.06 for flavor, 7.06 for taste and 7.66 for overall acceptability), while the lowest scores for flavor was obtained by ADJ-2 wine (4.26),for overall acceptability by ADJ-2wine (4.43) and for taste by ADJ-2wine (4.2). Score for clarity was highest in NCIM-3189 wine (7.4), followed by LI-3 wine (7.06); while the lowest score was obtained by both strains ADJ-1and ADJ-2 wines (4.53). NCIM-3189 (6.86), LI-3 (6.76) and LI-1 (6.73)wines obtained the highest score for colour which were *at par*,while, the lowest score was obtained by ADJ-2wine (4.16). In case of reverse rating for acidity and bitterness, the lowest scores for acidity was accorded to ADJ-1(4.1) and ADJ-2 (4.1) with the highest in NCIM-3189 wine (6.36). The highest bitterness reverse score was observed in LI-1 wine (6.36) with the lowest in ADJ-2wine (4.06) (Table 5).

Table (6) shows the means of combinations of wine types and attributes. Means of LI—1, 2, 3, 4 and NCIM 3189 wines were above the global mean. NCIM 3189 ranked top with the highest positive difference with the global means in all attributes. Attributes of ADJ-1 and ADJ-2 wines were below the global means.

Table (5): Sensory evaluation data of the developed wines

Wines	Colour	Acidity	Bitterness	Taste	Flavour	Clarity	Overall acceptance
LI-1	6.73	6.10	6.36	6.26	6.36	6.76	6.70
LI-2	6.13	6.16	5.56	6.13	6.16	6.53	6.80
LI-3	6.76	6.10	5.80	6.46	6.50	7.06	6.83
LI-4	5.80	5.53	6.10	5.56	6.00	6.23	6.53
ADJ-1	4.53	4.10	4.60	4.30	4.66	4.53	4.66
ADJ-2	4.16	4.10	4.06	4.20	4.26	4.53	4.43

NCIM3189	6.86	6.36	5.80	7.06	7.06	7.40	7.66
S Ed(±)	0.11	0.14	0.12	0.09	0.07	0.08	0.04
CD=(0.05)	0.26	0.31	0.24	0.16	0.11	0.16	0.09

Table (6): Adjusted means of the wines after sensory product characterization

Wines	Flavour	clarity	Colour	Overall acceptance	Acidity	Taste	bitterness
LI-1	6.37*	6.77*	6.73*	6.70*	6.10*	6.27*	6.37*
LI-2	6.00	6.23	5.80	6.53	5.53	5.57	6.10*
LI-3	6.50*	7.07*	6.77*	6.83*	6.10*	6.47*	5.80
LI-4	6.17	6.53	6.13	6.80*	6.17*	6.13	5.57
ADJ-1	4.67¥	4.53¥	4.53¥	4.67¥	4.10¥	4.30¥	4.67¥
ADJ-2	4.27¥	4.53¥	4.17¥	4.43¥	4.10¥	4.20¥	4.07¥
NCIM 3189	7.07*	7.40*	6.87*	7.67*	6.37*	7.07*	5.80

*Means are above global mean, ¥Means are below global means

IV. CONCLUSION

The present study identifies a yeast strain NCIM 3189 suitable for production of wines from jackfruit. Further works are needed to ascertain its efficacy in other fruits for alcohol production. However, before launching a commercial venture for the production of wine from identified fruits, there is need for a pre-industrialization pilot testing of the technology for assessing economic viability of such proposition. In addition to the crop source selected in the present study, we used many variable yeast strains to get the quality wine for each strain. There are still large number yeast strains available which can effectively be utilized for production of wine. Hence, further study in this field is warranted.

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