Table wine from tropical fruits utilizing natural yeast isolates

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Abstract An attempt was made to utilize few widely available tropical fruits to develop wine with the objective of comparing the fermentation efficiency (along with progress in fermentation) of two efficient yeast isolates with commercially available strain. Fruit wine from juices of fully ripe mango, jackfruit and pineapple alone and in blended combinations of all three fruit juice (2: 1: 2) was prepared using two different yeasts (Y4 and Y7) isolated from natural plain date palm juice and one standard *Saccharomyces cerevisiae* (MTCC-170) collected from IMTECH, Chandigar. Juices were extracted by using pectinase enzyme at 0.15–0.20 % of pulp. Changes in °Brix, titratable acid content, pH, total viable yeast count were recorded and rate of fermentation, sugar use efficiency were determined at every 24-hour interval up to the completion (6 days after inoculation) of fermentation. Considering all the quality parameter as well as fermentation efficiency, yeast isolate Y7 was found superior followed by Y4 as fermenting agent and pineapple juice as sole substrate found to be the most suitable medium for production of wine followed by fruit juice blending. In interpreting the efficacy of fruit and yeast in combination, pineapple juice inoculated with Y7 found to be the best in reducing the degree Brix to its lowest from initial 24 degree.

Keywords Fruit wine · Yeast isolate · Fermentation efficiency · Pectinase enzyme · *Saccharomyces spp.*

Introduction

Fruits, being good source of sugar, act as a potential substrate for production of fruit wine by fermentative processes. Besides, they are also good source of vitamins, minerals, aroma and colour. Fermentation is the oldest form of biotechnology where carbohydrate and other organic compounds are broken down under aerobic or anaerobic conditions (Potter 1978) by the microorganisms to get energy for their metabolic activities. Wine is made principally from grapes or extracts of other fruits (Joshi 1995; Modi 2009; Baidya 1990, 2010; Sharma et al. 2013) through fermentation by yeasts, producing significant quantity of ethyl alcohol depending upon the types of fruit wine. Different types of table wines e.g. dry, sweet and sparkling wines having alcohol content 8 to 15 % by volume (Anonymous 1995) are popular one throughout the world. Wine has prominent health benefits in controlling cardiovascular diseases (Sara 1998). Despite numerous research efforts, wine from fruits other than grapes has not been commercialized and gained popularity among the consumers. However, exploring the scope of wine fermentation from locally available fruits and establishment of wineries will reduce post harvest losses of excess fruits and lead to agricultural diversification and employment generation in rural India. Mango and pineapple comes under major fruits of West Bengal and the Agri-export zones of both these fruits were established in the state by the Government of India in 2002. Mango cultivar Bombai was used for fermentation that is comparatively cheaper and inferior in quality, therefore less acceptable as fresh. Although jackfruit is considered as underutilized fruit, it is also widely cultivated in West Bengal. Jackfruit has a very

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high sugar content and antioxidant activity, and less calories, which can be beneficial for health. Jackfruit pulps are usually consumed fresh as it has a low shelf life. Therefore, it is necessary to process the fruit into different food products to attain an economic value (Kumoro et al. 2012; Sharma et al. 2013). Reddy and Reddy (2009) reported that pectinase treatment increases the TSS content of clarified mango juice and also it improves the sensory attributes of prepared wine. Nidp et al. (2001) reported that *S. cerevisiae* was used to produce wine from pineapple juice after fermentation for 14 days while it took 21 days with *S. ellipsoideus*. However, Idise and Okiemute (2012) have successfully tried pineapple wine using natural yeast isolates. Wines produced by selected yeasts generally give better quality than those produced by spontaneous fermentation (Suchi and Modi 1997). The hot, humid climate that prevails is quite responsible for extremely brief shelflife of all the three fruits tend to adopt them for value addition in order to prevent spoilage.

Mango and jackfruit in general contains high amounts of total soluble solids (TSS), vitamins, minerals thus making it a suitable medium for the growth of wine yeasts. Also it can complement white wines which need to be prepared from white grapes, which in turn are a great worth when processed as raisins rather than wine (Beera et al. 2013). Keeping in view the above facts, attempts were made to prepare table fruit wines (dry) from mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*) and pineapple (*Ananas comosus*) juices with an aim to reduce huge wastage of these fruits during the peak harvest season using two different yeasts isolated from natural date palm juice and one standard yeast *Saccharomyces cerevisiae* (MTCC-170) collected from Institute of Microbial Technology (IMTECH, Chandigarh, India).

Materials and methods

Preparation of juice for fermentation Fruits of mango, jackfruit and pineapple were thoroughly washed with clean water before peeling and deseeding (in case of jackfruit and mango). Fruit pulps were extracted by using a pulper and then filtered through a strainer. During pulping, potassium meta-bisulphite was mixed with the pulp @ 200 mg/kg of pulp to prevent browning and to check the growth of undesirable micro-organisms. The ratio of jackfruit, pineapple and mango juice used in mixed fruit substrate was 1: 2: 2. As jackfruit juice had very strong aroma, its quantity was reduced by half as compared to other juices to have a balanced flavour in the mixture.

Treatment of fruit pulp with pectinase enzyme The pulp of each fruit was heated to 65°C for 15 min with continuous stirring, and then allowed to cool up to 40°C. Enzymatic

treatment of heated and cooled pulp with pectinase enzyme at 1.5 % for mango, 1.5 % for Jackfruit and 0.20 % for pineapple pulp) were mixed thoroughly and left for 4 h at ambient temperature (28–30°C), filtered, measured and clarity was observed.

Preparation of starter culture for inoculation of substrate

Yeast isolates Y4 and Y7 were selected out of 20 yeast isolates in the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalay on the basis of performance mainly efficiency to ferment (FE) molasses and grape juice to produce desired quality of wines while, the standard *Saccharomyces cerevisiae* strain (MTCC-170) was obtained from Institute of Microbial Technology (IMTECH), Chandigarh. The starter culture was prepared by growing the yeast isolates Y4, Y7 and MTCC170 in sterilized molasses broth (50 g l⁻¹) and incubated at 30 ± 1 °C for 24 h. Before mixing with inoculums or starter culture, TSS of molasses solution and all the fruit juices alone or in mixture was raised to 24°Brix by adding clarified cane sugar syrup and pH was adjusted to 4.0. Fresh culture @ 8 % (v/v) was used to inoculate the juices in conical flasks. Fermentation was conducted at room temperature (26–30 °C) in dark.

Evaluation of physico-chemical properties of wine

The observations on physico-chemical properties of fruit wines and fermentation efficiency of yeasts were recorded immediately after setting up of experiment and after every 24 h till the end of fermentation (6 days after inoculation) as indicated by no or minute change in °Brix. Bio-chemical parameters like TSS (by Digital Hand Refractometer, Model RHB-32, Made by Erma, Tokyo, Japan), pH (by Digital pH Meter manufactured by Systronics, Ahmedabad, India), percent acidity using 0.1 N NaOH (AOAC 1990), percent ethyl alcohol (w/w) and percent total sugar were analyzed following standard analytical methods (Ranganna 2000). Rate of fermentation was calculated as per standard formula (Baidya 1990) and yeast population/ml of substrates was counted by haemocytometer (made in Germany).

Fermentation efficiency (FE) Firstly the theoretical yield of alcohol (g %) was calculated by multiplying the amount of sugar (in gram) by 0.511 (La Grange et al. 2004) and then fermentation efficiency was calculated by the following formula:

$$\text{Fermentation efficiency (FE in\%)} = \frac{\text{Actual yield of alcohol}}{\text{Theoretical yield of alcohol}} \times 100$$

Data analysis The data were analyzed statistically by Factorial Completely Randomized Design (FCRD) to observe the performance of yeast isolates and fruit as sole or in

Fig. 1 Changes in TSS ($^{\circ}$ brix) in fermenting juice

combination on different quantitative parameters during fermentation. Statistical analyses consisted of data from quality analysis being subjected to analysis of variance (ANOVA) with MSTAT C statistical software and significant differences were determined by Tukey's test at the 5 % significance level.

Results and discussion

Changes in TSS A gradual decreasing trend of residual degree Brix was recorded at specific interval of 24-h throughout the fermentation process up to 6 days irrespective of treatment (Fig. 1) Indicating the use of fermentable sugar by all the three yeasts, however maximum decline in TSS was observed in case of y-7 followed by y-4 and MTCC170. Taking into

account the average efficiency of fruits to be used as fermenting medium, pineapple found to be the most suitable medium for fermentation bringing down the brix at the lowest i.e., 8.08 followed by mixed fruit Juice, mango and jackfruit. A gradual decrease in TSS is a typical nature of fermentation as it was observed by many workers like Ezeronye (2004) in their experiment with fruit wine.

Changes in total acidity It was evident from the result that although the acidity increased (Fig. 2) in most of the treatments (except in the case of mango - Y7 combination) there should have been a decrease in pH accordingly (Fig. 3). But it did not happen, indicating the increase of pH narrowly, in many cases. Similar observations were also made by Ethiraj and Suresh (1978) and Beera et al. (2013). However, although

Fig. 2 Changes in total acidity** (as citric acid) in fermenting juice. [**Initial total acidity (gram percentage as citric acid) of Mango =0.58 %, Jack fruit =0.64 %, Pineapple =0.49 %, Mixed fruit juice =0.55 % (before fermentation)]

Fig. 3 Changes in pH of fruit wines during fermentation

the acidity level was slightly erratic during the course of fermentation, at the end (144 h) it decreased in all treatment combinations. Titratable acidity of any fruit wine is an important parameters varying between 0.5 to 1 %. The acidity of fruit wine is dependent upon a no of factors as type of fruit, method of preparation and type of yeast used (Singh and Kaur 2009).

Rate of fermentation (fall of °Brix/24-hour) The rate of fermentation was quite higher during first 24-h then gradually it declined till the end except in case of mango and yeast combination, where rate was increased after 24-h and at the end it gradually decreased (Fig. 4). This might be due to the fact that yeasts have taken longer time to acclimatize leading to slow rate of fermentation at first

24-h. Till the end, the rates for all the treatments were in the range of 2.33–2.79 % per 24 h. From above experiments it might be concluded that Y7 (9.31 %) was the best producer of alcohol followed by Y4 (9.25 %) and MTCC170 (8.69 %). When fruit and yeast interaction was studied, Y7 gave highest yield of alcohol (9.90 % w/w) with mixed fruit, followed by Y4 with mixed fruit (9.60 %). Thus production of alcohol corroborated with the use of sugar. It indicated that the yeast Y7 was the fastest sugar fermenter. These results also reflect the typical trend of fermentation where due to low alcohol production initially, the rate of fermentation was higher and declined at later stage on account of production of more alcohol (Joshi and Sharma 1995). These findings are in accordance with the results of Beera et al. (2013) in mango wine.

Fig. 4 Changes in the rate of fermentation

Fig. 5 Fermentation efficiency (%) of selected yeasts in fermenting fruit juices at every 24-h interval

Changes in fermentation efficiency It went on increasing after 24-h and it mostly continued till 96 h except this increase

was noticed till 120 h (Fig. 5) in case of three combinations of mango-MTCC-170, jackfruit-Y4 and jackfruit-Y7. Highest

Table 1 Changes in yeast counts/ml at 24 h interval in fermenting fruit juices

Treatments	Fermentation time (in hour)						
	0 h. × 10 ⁷ Scale	24 h. × 10 ⁸ Scale	48 h. × 10 ⁸ Scale	72 h. × 10 ⁹ Scale	96 h. × 10 ⁸ Scale	120 h. × 10 ⁷ Scale	144 h. × 10 ⁶ Scale
y ₄	1.50	16.99	14.49	0.88	3.68	12.34	42.37
y ₇	3.88	10.13	37.68	1.28	6.48	6.43	35.41
MTCC170	6.57	6.44	6.50	2.39	1.32	4.52	7.39
S.Em (±)	0.08	1.23	1.31	0.11	0.65	0.48	2.67
CD at 5 %	0.24	3.59	3.84	0.34	1.93	1.41	7.81
Mango	3.33	4.80	18.04	1.38	9.97	11.49	87.47
Jackfruit	4.66	21.63	33.99	3.97	0.43	2.66	3.29
Pineapple	3.02	6.80	8.87	0.38	2.69	11.13	18.50
Mixed fruits	4.91	11.53	17.31	0.34	2.22	5.28	4.31
S.Em (±)	0.09	1.42	1.51	0.13	0.76	0.55	3.09
CD at 5 %	0.28	4.14	4.43	0.39	2.23	1.63	8.02
Mango × y ₄	2.50	4.06	15.73	1.21	8.92	15.93	122.66
Mango × y ₇	0.94	4.52	30.46	2.47	20.13	15.33	128.33
Mango × MTCC170	6.57	5.81	7.94	0.47	0.85	3.21	11.43
Jackfruit × y ₄	0.61	40.56	23.66	1.48	0.47	2.01	2.54
Jackfruit × y ₇	6.57	21.00	74.66	1.82	0.31	2.01	2.56
Jackfruit × MTCC170	6.80	3.32	3.65	8.61	0.51	3.95	4.28
Pineapple × y ₄	0.62	6.63	9.24	0.50	2.89	23.06	39.86
Pineapple × y ₇	2.05	6.05	9.00	0.34	2.58	6.20	8.29
Pineapple × MTCC170	6.39	7.74	8.39	0.30	2.60	4.14	7.34
Mixed fruit × y ₄	2.27	16.73	9.32	0.33	2.43	8.38	4.43
Mixed fruit × y ₇	5.96	8.97	36.60	0.50	2.91	2.19	2.46
Mixed fruit × MTCC170	6.50	8.90	6.03	0.19	1.32	6.78	6.3
S.Em (±)	0.16	2.46	2.63	0.23	1.32	0.96	5.35
CD at 5 %	0.49	7.18	7.68	0.68	3.86	2.83	15.62

fermentation efficiency was observed in mango-MTCC-170 treatment (99.22 %) followed by jackfruit-Y7 (98.92 %). Baidya (1990) observed almost similar level of fermentation efficiency in preparation of Jackfruit wine.

Changes in yeast counts/ml In the present study, the fermentation started after 10–12 h of inoculation with starter culture. From Table 1, it was evident that the yeast population at initial stage got increased and gradually declined at the later stage of fermentation. Maximum rise in yeast count was observed up to 48 h of fermentation for all the treatments. The results were found to be significant at 5 % level.

Changes in alcohol production (g %, w/w) It was evident from Table 2 that Mixed fruit-Y7 combination produced highest alcohol (9.90 g %) followed by mixed fruit-Y4 combination (9.60 g%) and pineapple-Y4 combination (9.49 g %). Kumoro et al. (2012) prepared jackfruit wine within 7 days with 12–13 % alcohol. Although Sharma et al. (2013) suggested 14 days to prepare jack wine with 11–13 % alcohol.

Table 2 Rate of alcohol production (g% w/w) of fruit wine during fermentation

Treatments	Fermentation time (in hour)					
	24	48	72	96	120	144
y ₄	4.60	6.56	8.07	8.81	9.08	9.25
y ₇	4.89	7.59	8.84	9.22	9.26	9.31
MTCC170	4.17	6.26	7.78	8.45	8.61	8.69
S.Em (±)	0.02	0.03	0.03	0.05	0.03	0.03
CD at 5 %	0.06	0.12	0.09	0.17	0.10	0.09
Mango	1.10	4.07	6.91	8.29	8.88	9.07
Jackfruit	8.06	8.66	8.71	8.75	8.77	8.80
Pineapple	4.35	6.97	8.42	9.10	9.12	9.27
Mixed fruits	4.71	7.52	8.88	9.17	9.16	9.19
S.Em (±)	0.02	0.03	0.03	0.06	0.04	0.03
CD at 5 %	0.07	0.12	0.11	0.20	0.12	0.11
Mango × y ₄	1.03	3.50	5.97	7.71	8.69	9.11
Mango × y ₇	1.60	5.66	8.12	8.94	9.09	9.12
Mango × MTCC170	0.67	3.06	6.64	8.23	8.87	8.98
Jackfruit × y ₄	7.91	8.70	8.70	8.73	8.76	8.79
Jackfruit × y ₇	8.02	8.68	8.77	8.81	8.82	8.85
Jackfruit × MTCC170	8.24	8.61	8.66	8.71	8.71	8.78
Pineapple × y ₄	5.17	7.14	8.69	9.23	9.29	9.49
Pineapple × y ₇	4.95	8.00	8.77	9.24	9.25	9.38
Pineapple × MTCC170	2.92	5.76	7.80	8.83	8.83	8.94
Mixed fruit × y ₄	4.27	6.92	8.93	9.59	9.59	9.60
Mixed fruit × y ₇	4.99	8.03	9.72	9.88	9.88	9.90
Mixed fruit × MTCC170	4.86	7.63	8.00	8.03	8.03	8.08
S.Em (±)	0.04	0.05	0.06	0.11	0.07	0.06
CD at 5 %	0.12	0.15	0.19	0.38	0.21	0.19

Sensory evaluation scores of wine correlated to the presence of higher alcohols (Reddy and Reddy 2009).

Conclusion

The present research work is accomplished to compare the efficacy of naturally isolated yeasts with the established strain (Y33) of *S. cerevisiae* collected from IMTECH, Chandigarh for the preparation of wine out of locally available fruits and the performance of both Y4 and Y7 was comparatively better than Y33. On the basis of these studies, it can be concluded that good quality table wines with 8 to 10 percent of alcohol can be prepared from mango, jackfruit and pineapple and juice combination of these fruits using different natural and standard yeast isolates. It takes at least 6 days to complete fermentation and at this stage, mixed fruit-Y7 combination produced highest alcohol (9.90 g %) followed by mixed fruit-Y4 (9.60 g %) and pineapple-Y4 combination (9.49 g %). Wine prepared from mentioned fruits can be a very good substitute for the value addition of these particular fruits.

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