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Evaluation of yeast and acetic acid bacteria for vinegar production from overripe fruits pineapple, banana, and papaya

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Abstract
Isolation of yeast and acetic acid bacteria from pineapple, banana, papaya, apple, orange, custard apple, grapes, and pomegranate juice were attempted and microbial isolates were identified, and efficient isolates were screened for vinegar production. Among the isolates, UASB Y-1 scored highest concerning alcohol production, and acetic acid bacteria UAS Aab-2 produced the highest percent of acetic acid in a preliminary screening of individual fruit juices and their combination. The acetic acid percent was highest in vinegar produced from pineapple juice inoculated with UAS Aab-2.

Keywords: Vinegar, yeast, bacteria, fruit juice, and evaluation of isolates

Introduction
India is one of the countries which is mainly based on agriculture. In fruit production, India ranks second next to China. It shares 12.5% of the world's production. India covers an area of 6506,000 ha with a production accounting to 97358,000 MT and productivity is 14.96 MT/Hectare (Anon., 2018) [1]. Since fruits are produced on a large scale there are post-harvest losses from field to consumer level. Post-harvest loss accounts for 32% in India. To avoid these losses, various processing methodologies can be applied for the value addition of these harvested fruits. Among them, vinegar production from overripen fruits is one of the ways to reduce post-harvest losses. Vinegar is an important preservative and condiment and it is being produced for centuries. It is produced through the action of acetic acid bacteria on a dilute solution of ethyl alcohol derived from yeast fermentation. Acetic acid is the predominant flavouring and antimicrobial component in vinegar (Marshall *et al.*, 2000) [3]. Vinegar is made from a wide variety of fruits, pineapple peel, and cereals. Over the past several decades there has been a growing trend towards adding value to raw agricultural by-products. All commercial vinegar is used primarily in the food processing industry as a preservative. Pineapples are produced in many tropical regions of Africa where rainfall is adequate and moisture is consumed locally. It is an important luxury fruit of tropical regions. In India Pineapple occupy an area of 103,000 ha, with a production of 1706,000 MT in 2017- 2018 (Anon., 2018) [1] there is 29% of post-harvest loss in the fruits after harvest. Vinegar is an inexpensive commodity; therefore, the economic considerations require a relatively low-cost raw material like pineapple peel which can be used in vinegar production. Banana (*Musaceae musa)* is a fast-growing herbaceous perennial arising from underground rhizomes. It is being cultivated in an area of about 884,000 ha, with production is 30808,000 MT and post-harvest loss was 15% in 2018. It contains Vitamin C (10.74mg) and a high protein, which includes three of the essential amino acids. Ripened banana is useful in acidity. Heart burns resulting from consumption of tea may be neutralized (eat two before taking tea). It has a total amount of Vitamin C or ascorbic acid (10.74mg). Papaya (*Carica papaya* L.) is a good source of vitamins, dietary fibre, and minerals and provides flavor, aroma, and texture to the pleasure of eating. It is being grown in an area of 138,000 ha and production is 5989,000 MT post-harvest loss was 46%. Nutritionally, papaya is a good source of calcium and an excellent source of vitamin A and C (Nakasone and Paull, 1998) [5]. Fully ripened papaya fruits are usually eaten fresh as the enzymes in the fruit produce calm, soothing feelings in the stomach. So, by keeping these facts the work was carried out on evaluation of yeast and acetic acid bacteria for vinegar production from Pineapple, Banana, and Papaya fruits.

Material and Methods

Isolation of yeast and acetic acid bacterial isolates from different fruit sources

Different fruits like pineapple, banana, papaya, grapes, pomegranate, orange, and apple were collected for the isolation of yeast and acetic acid bacteria. Yeast and acetic acid bacteria were isolated from fruit juices by enrichment broth containing cultures and then by standard plate count method using Davis agar medium (Sobia *et al*., 2007) [6] and Ethanol agar medium (Moryadee and Wasu, 2008) [4] respectively. The strains of *Saccharomyces cervisiae* UCD 522 and *Acetobacter aceti* MTCC 2945 were procured from Post-Harvest Technology Scheme and were used as a reference culture.

Evaluation of yeast and acetic acid bacteria for vinegar production from Pineapple, Banana, Papaya fruits

The unutilized, overripe pineapple, banana, and papaya fruits are cut into small pieces and grounded using a mixer grinder to produce fruit slurry and it was mixed by adding water in different ratios (w/v) and homogenized. This was filtered using muslin cloth manually. The clear juice was collected and used for further experiments. A loop full of inoculum of yeast culture was transferred to a conical flask containing Davis broth. The flask was kept overnight for yeast growth in the broth. This yeast culture was then added to 300 ml fruit juice in a 500ml flask. This culture was used at 5 percent (v/v) for fermentation. Acetic acid bacterial starter culture was prepared similarly but here broth used was ethanol broth. The isolated yeast isolates were evaluated for maximum alcohol production using respective fruit juice. The yeast isolates were inoculated into the fruit juice and allowed for anaerobic alcohol fermentation for a week and estimated the alcohol percent. Fruit juice + UASB Y-1, Fruit juice + UASB Y-2, Fruit juice + UASB Y-3, and Fruit juice + (Reference yeast - *Saccharomyces cerevisiae* UCD 522). The isolated acetic acid bacterial isolates including reference strain have to be evaluated for the production of maximum vinegar production from fruit juice based on their efficiency of the acetic acid production. The bacterial isolates were inoculated into the fruit wine and incubated under aerobic conditions for acetic acid fermentation for six days and then acetic acid content was determined by titration with 0.1N NaOH against Phenolphthalein as an indicator. Fruit juice + UAS Aab-1, Fruit juice + UAS Aab-2, Fruit juice + UAS Aab-3, and Fruit juice + (Reference acetic acid bacteria- *Acetobacter aceti* MTCC2945).

Treatment details

T $_1$= Pineapple juice alone (300 ml)
T $_2$= Banana juice alone (300 ml)
T $_3$= Papaya juice alone (300 ml)
T $_4$= Pineapple + Banana juice (150:150 ml)
T $_5$= Pineapple + Papaya juice (150:150 ml)
T $_6$= Banana + Papaya juice (150:150 ml)
T $_7$= Pineapple + Banana + Papaya juice (100: 100:100 ml)

For these treatments yeast and acetic acid bacterial isolates were inoculated for evaluation of higher ethanol and acetic acid content.

Result and Discussion

The quality of vinegar depends on the composition of raw material used and also differs with different strains used for fermentation. The experimental results on isolation and evaluation of yeast and acetic acid bacterial isolates from different sources for Pineapple, Banana, and Papaya vinegar production and organoleptic evaluation of the developed vinegar. All the strains of yeasts were screened for alcohol production using fruit juices like Pineapple(T1), Banana(T2), Papaya(T3), Pineapple + Banana(T4), Pineapple +Papaya (T5), Banana + Papaya(T6), Pineapple + Banana +Papaya (T 7) which is furnished in (Table 1). The alcohol production was higher in the case of Pineapple fruit juice (8.1%) which was produced by reference strain *Saccharomyces cerevisiae* UCD 522 followed by isolated pineapple yeast strain, UASBY-1 (7.9%). The lower alcohol (6.5%) was produced in blended fruit juice Banana + Papaya (Table 1). The variation in the production of alcohol by different yeast strains may be due to the variation in their rate of utilization of sugar in fermentation medium and alcohol tolerance capacity. This view is in confirmation with the results of Chaudhari and Chincholkar (1996) [2] who reported that among 30 yeast strains; a strain was able to ferment 15% total sugars in molasses to ethanol (15g/l)

Table 1: Evaluation of different yeast isolates Alcohol production in different fruit juice

Isolates	Treatments							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean**
UASBY-1	7.9	7.6	7.5	7.6	7.3	6.5	7.5	7.41
UASBY-2	7.6	7.5	7.4	7.3	7.2	6.7	7.6	7.32
UASBY-3	7.4	7.2	7.3	7.5	7.6	7.1	7.6	7.38
Reference strain	8.1	7.8	7.9	7.9	7.8	7.7	8.0	7.88
Mean	7.75	7.52	7.52	7.57	7.47	7.00	7.67	
Source	S. Em	CD 0.5%						
Strains(S)	0.043	0.123						
Treatments (T)	0.577	0.163						
Interaction (T×S)	0.11	0327						

Note: UASB Y_1 = Pineapple yeast, UASB Y_2 = Banana yeast, UASB Y_3= Papaya yeast, and Reference strain = *Saccharomyces cerviceae* UCD 522

All the isolated strains along with reference strain *Acetobacter aceti* MTCC 2945 were evaluated for maximum acetic acid production which was presented in table 2. The acetic acid production was higher (4.4%) in pineapple fruit wine which was produced by reference strain *Acetobacter aceti* MTCC 2945 followed by (4.2%) in pineapple fruit juice by isolated banana acetic acid bacterial strain. The lower (3.2%) acetic acid content was observed in Pineapple+ Papaya fruit wine by isolated Pineapple acetic acid bacteria, which is presented in Table 2. Thus the variation in the production of acetic acid by

different acetic acid bacterial strains may be due to the variation in the utilization of sugar and alcohol in the fermentation medium and acetic acid tolerance capacity by acetic acid bacteria. This is in confirmation with the results of Moryadee and was (2008) [4] who reported that *Acetobacter aceti* isolate No. 37 from rambutan gave the highest acetic acid% after seven days of fermentation.

Table 2: Evaluation of different Acetic acid bacterial isolates on acetic acid production in different fruit juice.

Isolates	Treatments							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**Mean**
UAS Aab1	4.1	3.9	3.6	3.8	3.2	3.3	3.6	3.9
UAS Aab2	4.2	3.7	3.7	3.7	3.6	3.5	3.5	3.7
UAS Aab3	3.9	3.8	3.5	3.6	3.4	3.3	3.5	3.5
Reference strain	4.4	4.2	3.8	3.9	3.8	3.7	3.8	3.94
Mean	4.15	3.9	3.65	3.75	3.5	3.45	3.6	
Source	S.Em	CD 0.5%						
Strains(S)	0.043	0.123						
Treatments (T)	0.577	0.163						
Interaction (T×S)	0.115	0.327						

Note: UAS Aab $_1$= Pineapple Aab, UAS Aab $_2$= Banana Aab, UAS Aab $_3$= Papaya Aab, and Reference strain = *Acetobacter aceti* MTCC 2945. (Aab= Acetic acid bacteria)

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