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Standardization of sugar concentration for vinegar production

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Abstract
The standardization of protocol was formulated for vinegar production from pineapple juice through microbial fermentation by yeast and acetic acid bacteria. In different levels of sugar concentration 25° Brix sugar concentration is optimal for alcohol production and 15° Brix is optimal for acetic acid production. The overall result showed that the vinegar produced by yeast UASB Y-1 and acetic acid bacteria UAS Aab-2 at 15º Brix concentration is the optimum level for vinegar production.

Keywords: Sugar concentration, yeast, bacteria, vinegar

Introduction
Vinegar is a living ingredient formed through the process of fermentation. The term "vinegar" refers to the two-step process of fermentation from carbohydrate to alcohol to acetic acid. Sugar is converted into alcohol, which intern fermented into vinegar. Not all acetic acids are vinegar, although all vinegar is made from acetic acid. Acetic acid is the main flavouring and antimicrobial constituent in vinegar (Marshall *et al.*, 2000) [3]. A sugar concentration in general range from 10-18 percent is ideal for making vinegar stock. This is because concentrations of alcohol at 9-12 percent are considered optimal for vinegar production.

Material and Methods
The experiment is related to the standardization of protocol for vinegar production. Here pineapple fruit source is used for standardization which was done to know the optimum range of sugar concentration for higher acetic acid production. Fruits are cut into small pieces and grinded using a mixer grinder to produce fruit slurry and it was mixed by adding water in different ratios (w/v) and homogenized. It is done by adjusting different sugar concentrations by adding cane sugar to fruit juice at 10° Brix, 15° Brix, 20° Brix, and 25 ° Brix. Here cane sugar is added to fruit juice and adjust the sugar content for 10°,15°, 20°, and 25° Brix and allowed for yeast and acetic acid bacteria for the fermentation of fruit juice. 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 cultures were 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.

Organisms used in the study
Yeasts,
UASBY-1 =Pineapple yeast isolate
Reference yeast = *Saccharomyces cerevisiae* UCD 522

Acetic acid bacteria
UAS Aab-2 = Banana acetic acid bacterial isolate
Reference bacteria = *Acetobacter aceti* MTCC 294**.**

Result and Discussion

Concerning maximum alcohol production, significant differences were noticed between the strains and treatments which are presented in Table 1. The higher (10.2%) alcohol production was recorded in 25° Brix treatment by reference strain *saccharomyces cerevisiae* followed by (9.9%) was recorded in 25° Brix treatment by isolated strain Pineapple yeast. The lower (5.4%) alcohol production was noticed in 10° Brix treatment by isolated strain Pineapple yeast. In this present investigation, the data revealed that there was a gradual increase in alcohol due to the addition of sugar into fruit juice. These results are in comparison with the maximum production of ethanol at optimum natural sugar concentration (10%) of guava pulp was 5.8(w/v) by isolate 2, which was slightly more than the quantity of ethanol produced by *Saccharomyces cervisiae* (5.0%) and isolate-1 (5.3%) (Srivastava and Kumar, 1993) [6] And at 20° Brix reported by (Seyram *et al.*, 2009) [5].

Table 1: Effect of different sugar concentrations on alcohol production from pineapple juice.

Isolate	Different concentrations of sugar				
	10° Brix	15° Brix	20° Brix	25° Brix	Mean
UASBY-2	5.4	8.0	8.3	9.9	7.9
Reference strain	5.5	8.1	8.5	10.2	8.07
Mean	5.45	8.05	8.4	10.05	
Source	S. Em+	CD 0.05%			
Strains (S)	0.0577	0.173			
Treatments (T)	0.0816	0.244			
Interaction (S×T)	0.1155	0.346			

Note: UASB Y_1 = Pineapple yeast isolate, Reference strain = *Saccharomyces erevisiae* UCD 522

Table 2 indicates that significant differences were noticed between strains and treatments. The higher (4.2) acetic acid content was recorded in 15° Brix treatment by reference strain *Acetobacter aceti* followed by 3.38 in 15° Brix treatment. The lower acetic acid was recorded in 25° Brix by isolated banana acetic acid bacterial strain. Raspor and Goranovic (2008) [4] cited that alcohol used for a certification should have low ethanol content (7 to 9%). Gullo *et al*., 2005 [1] reported that the greatest hurdle to acetic acid bacteria growth is the high sugar concentration. And also, this view is in confirmation with Horiuchi *et al*. (2000) [2] who reported that acetic acid yield was related to the amount of sugar consumed by yeast cells.

Table 2: Effect of different sugar concentrations on the acetic acid production from pineapple juice.

Isolate	Different concentrations of sugar				
	10° Brix	15° Brix	20° Brix	25° Brix	Mean
UAS Aab2	3.33	4.10	3.23	3.00	3.23
Reference strain	4.01	4.20	3.56	3.20	3.74
Mean	3.67	3.79	3.39	3.10	
Source	S. Em+	CD 0.05%			
Strains (S)	0.027	0.085			
Treatments(T)	0.038	0.120			
Interaction(S×T)	0.056	0.172			

Note: UAS Aab_2= Banana Aab: Reference strain = *Acetobacter aceti* MTCC 2945. (Aab= Acetic acid bacteria)

The variation in the production of alcohol by different yeast strains may due to the variation in their rate of utilization of sugar in fermentation medium and alcohol tolerance capacity. Among the different concentrations of sugar, the highest alcohol (10.2%) was observed by *Saccharomyces cerevisiae* UCD 522 in 25° Brix concentration followed by UASBY 1(9.9%). In this present investigation, the data revealed that there was a gradual increase in alcohol due to the addition of sugar into fruit juice. These results are in comparison with the maximum production of ethanol at optimum natural sugar concentration (10%) of guava pulp was 5.8(w/v) by isolate 2, which was slightly more than the quantity of ethanol produced by *Saccharomyces cerevisiae* (5.0%) and isolate-1 (5.3%) (Srivastava and Kumar, 1993) [6] And at 20° Brix reported by (Seyram *et al.*, 2009) [5].

The higher (4.2) acetic acid production was recorded in 15°Brix by *Acetobacter aceti* and the lower (3.0) was recorded in 25° Brix by UAS Aab2. This may be due to higher sugar and alcohol in fermentation sources for acetic acid bacteria. Because Raspor and Goranovic (2008) [4] cited that alcohol used for acetification should have low ethanol content (7 to 9%). Gullo *et al*., 2005 [1] reported that the greatest hurdle to acetic acid bacteria growth is the high sugar concentration. And also, this view is in confirmation with Horiuchi *et al*. (2000) [2] who reported that acetic acid yield was related to the amount of sugar consumed by yeast cells.

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