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Standardization of pH 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 pH, a higher percentage of alcohol produced from the treatment which was standardized to 7.5 pH level and 5.5 pH is optimal for acetic acid production.

Keywords: PH, alcohol, acetic acid

Introduction
Vinegar is an important preservative and condiment and it is being produced for centuries. Vinegar means sour wine, it can be made from anything containing sugar, such as fruit. 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 flavoring and antimicrobial component in vinegar (Marshall *et al.*, 2000). Vinegar is also produced from fermented cider, fruit juices, or other fermented alcoholic solution derived from barley malt, hydrolyzed cereals, and starches. In general, the pH is an important quantity that reflect the chemical conditions of a solution. The pH of eight varieties of different fruit juices ranged from 3.10 to 3.70 which were used for the preparation of red wines was reported by Suresh *et al*. (1983) [6]. Seyram *et al*., (2009) [5] reported that increasing the production of acetic acid may the cause of the decrease in pH of pineapple vinegar to 2.8.Segun (2012) [4] reported that vinegar produced from sweet orange at 14 days fermentation gave pH (3.46) and total solid content (8.70%) values of no significant difference. The colour, aroma, taste and overall acceptability of vinegar from sweet orange peels fermented for 4, 6 and 14 days were not significantly different. There was a significant correlation between acetic acid and pH as well as total acidity. So, the objective of the study is to standardize the pH for vinegar production by yeast and acetic acid bacteria from pineapple.

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 pH levels for higher acetic acid production. Unutilized, overripe post-harvest fruits were used for the study. Fruits were 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. It was done by adjusting fruit juice at different pH levels, which was done by adding acid/base to fruit juice at 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 pH and allowed for fermentation. And the experimental set was the same as done previously. A loop full of inoculum of yeast culture was transferred to a conical flask containing Davis broth. The flasks were 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.

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

Acetic acid bacteria
UAS Aab-2 = Pineapple acetic acid bacterial isolate
Reference bacteria = *Acetobacter aceti* MTCC 2945

Result and Discussion
Alcohol production (%) at different pH levels
At different pH levels, a significant difference was noticed between treatments and strains for alcohol production which was presented in Table 1. The higher (10.0) alcohol production was recorded in 7.5 pH by reference strain *Saccharomyces cerevisiae* followed by (9.6%) alcohol production by isolated Pineapple yeast strain. The lower (5.0) alcohol production was recorded in 8.5 pH treatment by isolated pineapple yeast strain. This view is in confirmation with Olasupo and Obayori (2003) [3] who reported that the pH gradually decreased from an initial pH of 7.3 to 3.5 at the end of the fermentation.

Table 1: Effect of different levels of pH on alcohol production from pineapple juice

Isolate	Different levels of pH						
	3.5	**4.5**	**5.5**	**6.5**	**7.5**	**8.5**	**Mean**
UASBY1	6.40	7.20	8.10	8.5	9.6	5.00	7.46
Reference strain	6.60	7.30	8.20	8.8	10.0	5.20	7.76
Mean	6.50	7.50	8.10	8.65	9.8	5.10	
		Source	S.Em+	CD 0.05%			
		Strains (S)	0.047	0.137			
		Treatments (T)	0.081	0.238			
		Interaction (S×T)	0.115	0.337			

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

Acetic acid content (%) at different pH levels
A significant difference was noticed between treatments and strains for acetic acid production which was presented in Table 2. The higher (4.29) acetic acid production was recorded in 5.5 pH treatment by reference strain *Acetobacter aceti* followed by (4.14) by isolated banana acetic acid bacteria. The lower (3.12) acetic acid production was noticed in the 3.5 pH treatment by isolated strain banana acetic acid bacteria**.** Acetic acid production is one of the parameters to test the efficiency of acetic acid bacterial strains since acetic acid is a major acid in vinegar. Thus, the variation in the production of Acetic acid by different acetic acid bacterial isolates 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 and also due to the variation in the pH levels. This is in confirmation with the results of Matsushita *et al*. (1997) [2] reported that acetic acid bacteria grow optimally at pH 4.0 to 6.5. and the optimum growth requirements of *Acetobacter spp,* depend on the pH of the medium. At a pH value below 3.5 growth does not occur.

Table 2: Effect of different levels of pH for acetic acid (%) production from pineapple fruit juice

Isolate	Different levels of pH						
	3.5	**4.5**	**5.5**	**6.5**	**7.5**	**8.5**	**Mean**
UAS Aab2	3.12	3.19	4.14	3.23	3.12	3.16	3.32
Reference strain	3.26	3.31	4.29	3.43	3.30	3.38	3.49
Mean	3.19	3.25	4.21	3.33	3.21	3.27	
		Source	S. Em+	CD 0.05%			
		Strains (S)	0.0236	0.0688			
		Treatments (T)	0.0408	0.1191			
		Interaction (S×T)	0.0577	0.1685			

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

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