Production of Vinegar from Pineapple Peel Wine Using *Acetobacter* Species

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***Abstract*—**Pineapple peel is generally considered as waste material and do contribute to environmental pollution upon decay. The need to convert the peel into a value added commodity became imperative. Vinegar, a product of two-stage fermentation was produced from Pineapple peel wine. The wine was produced from the peels' juice using *Saccharomyces cerevisiae* through the process of fermentation and further oxidized to vinegar (acetic acid) using *Acetobacter* species. The wine had a total alcohol content of 10.8%. The vinegar produced had a pH value of 3.6, a total solids value of 10.2% and titratable acidity of 0.24 g/ml (lactic acid) and 0.16g (acetic acid). *Acetobacter*_species isolated from honey appeared whitish with granulated surfaces after 48 h with formation of slightly clear zones observed around the colonies. The vinegar contained a total acidity of 3% (acetic acid). Consequent upon this research, materials like pineapple peel considered generally as waste can be bio converted into important value-added materials thus aiding environmental safety.

Keywords*—Acetobacter* species, Pineapple peel, Vinegar, pH, Wine.

I. Introduction

THE juice in pineapple has been fermented into an alcoholic beverage commonly called wine. Pineapple wine is a non-vintage wine made from the juice of pineapple, which is produced and fermented in similar manner as grape wines. Fermentation of the pineapple juice takes place in temperature controlled vats and is stopped at near dryness. The result is a soft, dry, fruity wine with an unmistakable pineapple bouquet. It is a type of fruit wine popular in Hawaii (Samson, 1998).

Vinegar has been known worldwide as a seasoning or food preserving agent. Vinegar is defined as "a liquid fit for human consumption, produced from suitable raw materials of agricultural origin containing starch, sugars, or starch and sugars by the process of double fermentation, alcoholic and acetous, containing a specified amount of acetic acid" (Pooja and Soumitra, 2013). Vinegar, a traditional acidic condiment, is widely produced from rice, malt, apples, wine, molasses, dates, sorghum, apples, pears, grape, berries, melons, coconut, honey, beer, maple syrup, potatoes, beets, malt grains and whey and various other agricultural materials (Frazier and Westhoff, 2002).

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Vinegar production ranges from traditional methods employing wood casks and surface culture to submerged fermentation (Morales *et al*., 2002).Vinegar fermentation is essentially a two stage process, firstly the anaerobic conversion of fermentable sugars to ethanol by yeasts, usually *Saccharomyces* species, and secondly the aerobic oxidation of ethanol to acetic acid by bacteria, usually *Acetobacter* species. Acid yield improvements can be achieved using high rates aeration during continuous production (Adams, 1998). *Acetobacter* is a genus of acetic acid bacteria characterized by the ability to convert alcohol, C_2H_5OH, (ethanol) to acetic acid CH_3CO_2H, in the presence of air by oxidation.

There are several species within the genus, and there are other bacteria capable of forming acetic acid under various conditions. *Acetobacter* are of particular importance commercially because, they are used in the production of vinegar. *Acetobacter* species can destroy wine which it infects by producing excessive amounts of acetic acid. Acetic acid bacteria are acid tolerant, growing well below pH 5.0, although the pH optimum for growth is 5.4 to 6.3 (Madigan and Martinko, 2005).

The chemical and organoleptic properties of vinegar are a function of the starting materials and the fermentation method. Acetic acid, the volatile organic acid that identifies the products as vinegar, is responsible for the taste flavor and pungent, lifting odor of vinegar. However acetic acid should not be considered synonymous with vinegar (Nester *et al*., 2003).

In the United States a vinegar product contains a minimum of 4% acidity. European Countries have regional standards for vinegar produced or sold in the area. While distilled vinegar are generally 4% to 7% acetic acid whereas cider and wine vinegar are 5% to 6% acetic acid (Morales *et al*., 2002)

Vinegar has found some uses due to its anti- infective properties, anti-tumour activities; control of blood glucose and for salad dressing. The accumulations of pineapple peel residue do not only contribute to environmental pollution, but also reduces its value-added potential. This study was aimed at determining whether or not vinegar; a value added product can be produced from pineapple residue.

II. Materials And Methods

A. Sources

Pineapple peel wine was produced using *Saccharomyces cerevisiae*. The honey from which *Acetobacter* species was isolated was obtained from Jalingo, Taraba State, Nigeria respectively.

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B. Medium composition

A compounded selective glucose, yeast extract, calcium carbonate, ethanol (G.Y.C) agar medium was used for the isolation of *Acetobacter* species. This medium contained glucose (5.0g), yeast extract (1.0g), calcium carbonate (3.0g), Agar (2.5g) and ethanol (7%).

All components of the medium except ethanol were added to 93 ml of distilled water and mixed thoroughly. The medium was gently heated and brought to boil on a hot plate after which it was autoclaved for 15 minutes at 15-psi. Upon cooling to about 45^{o}C, 7% absolute ethanol was added and poured into sterile Petri dishes to gel.

C. Isolation of Acetobacter species

From the honey sample, 1 ml was added to 9 ml of sterile normal saline. From the stock, 1 ml was serially diluted to the dilution of 10^{-4}. From the last dilution, 0.5 ml was poured on the G.Y.C agar plates and streaked. The inoculated plates were incubated at 30°C for 48 hr. Colonies that appeared on the medium were sub-cultured to obtain pure cultures.

Biochemical characterization of the isolate was done according to standard methods.

D. Oxidative Fermentation of the Wine to Vinegar

A 650 ml volume of the racked wine in a 1 litre Erlenmeyer flask was inoculated with pure culture of *Acetobacter* species. Sterile cotton wool was used to cover the mouth of the conical flask to allow for the aeration of the broth during the fermentation. This was monitored at intervals for the period of 12 days (Fellows, 1997)

III. Results

TABLE I
THE CHEMICAL COMPOSITION OF THE PINEAPPLE PEEL WINE ANALYZED

Pineapple Peel Wine	Composition/characters
Specific gravity	1.010
Total Solids	10.6
pH	3.8
Total acidity (lactic acid)	0.090
Total acidity (acetic acid)	0.06
Alcohol contend (%)	10.0
Colour	Light yellow
Flavour	Pineapple bouquet
Taste	Harsh

TABLE II
BIOCHEMICAL IDENTIFICATION OF THE ISOLATE

***Acetobacter* species**	***Characteristics***
Growth at 30^{o}C	+
Growth at 37^{o}C	-
Motility	+
Gram reaction	-
endospore stain	-
catalase	+
Oxidase	-
Colonial appearance	White with granulated surfaces with zones of clearance around colonies

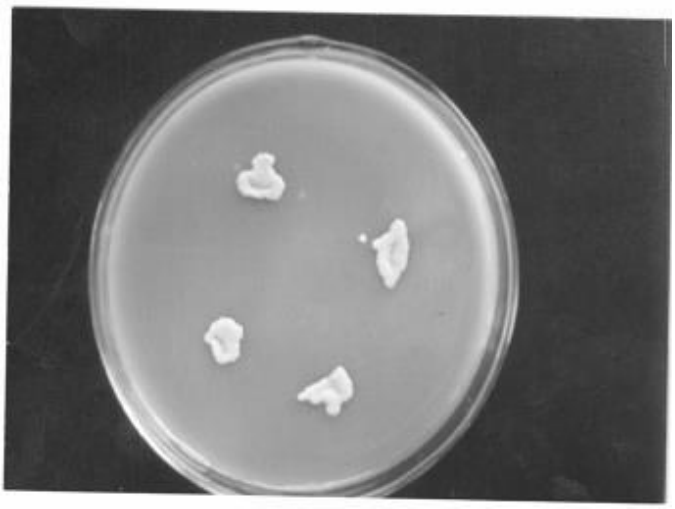

Fig. 1 Colonies of *Acetobacter* colonies on composed (G.Y.C.E) Agar medium. Inoculation into cut portions on agar

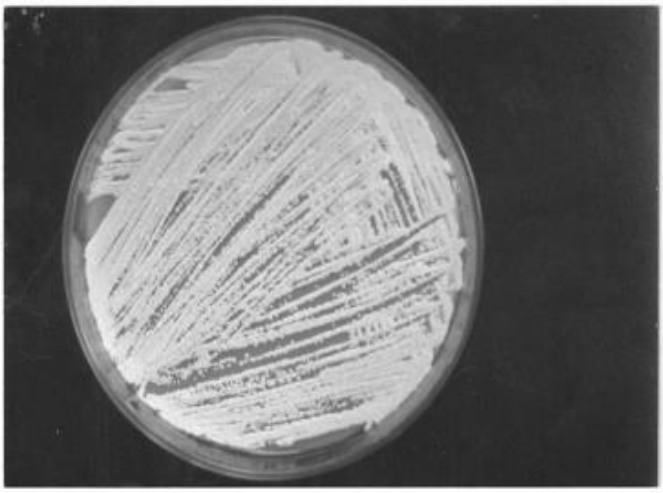

Fig. 2 *Acetobacte*r colonies on (G.Y.C.E) Agar upon streaking

TABLE III
TOTAL ACIDITY AND PH OF THE VINEGAR PRODUCED.

	Total titratable acidity		
Days	**pH**	**Lactic Acid**	**Acetic Acid**
0	3.8	0.24	0.16
2	3.7	0.23	0.15
4	3.7	0.24	0.16
6	3.6	0.23	0.16
8	3.6	0.24	0.16
10	3.6	0.23	0.16
12	3.6	0.24	0.16

IV. Discussion

Acetobacter species was isolated from honey and identified based on biochemical and morphological characteristics. The *Acetobacte*r isolate like any other *Acetobacter* strains, was endospores negative, catalase positive and oxidase negative (Adams and Moss, 1999). Based on colonial morphology, the colonies appeared whitish on the medium with granulated surfaces. The bacterium appeared to lift the calcium carbonate particles to the surface of the colonies with zones of clearance formed around the colonies. This could have been so because

when *Acetobacter* colonies form enough acetic acid from the ethanol, the calcium carbonate around the colonies dissolves, forming a very distinct clear zone as reported by Madigan and Martinko, (2005) (Refer to Fig. 1 and 2).

The vinegar produced had a pH of 3.6 and 3% total acidity (acetic acid). This fell below the 5% to 6% acetic acid for wine vinegars and 4% to 7% total acidity for distilled vinegars as reported by Morales *et al*., (2002). This could be attributed to interruption of air supply during the oxidation process as acetic acid bacteria are reported to be very susceptible to air interruption during oxidative processes (Madigan and Martinko, 2005). The variation in percentage acetic acid content of the pineapple peel wine vinegar could also be as a result of incomplete oxidation of the ethanol to acetic acid. It could also be dependent on the wine type used.

The vinegar had characteristic pineapple bouquet, a harsh (tart) taste and a light yellow colour implying that pineapple peel which conventionally could be regarded as waste, can be converted into value-added commodity, thus, facilitating environmental safety.

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