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Vinegar Production using *Acetobacter aceti* Isolated from Over-ripped Bananas

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Abstract

The demand for food and food preservatives rises with population growth, and post-harvest fruit losses are widespread in emerging nations, particularly in Nigeria. In order to avoid the usage of chemically produced preservatives, it is essential in today's society to convert waste into vinegar, which can be utilized as an organically created preservative. In essence, vinegar production is a two-phase fermentation process that includes fermentation to alcohol and acetic acid, respectively. In this study, Baker's yeast (Saccharomyces cerevisiae) was introduced into banana worts with a view to fermenting into alcohol (Ethanol) for 7 days, after which acetic acid bacterium (Acetobacter aceti) isolated from over-ripped bananas was inoculated into the produced alcohol to form vinegar. Analyses carried out on the produced vinegar were done using acetic acid and Fourier transform infra-red (FTIR) assays, which gave 1.4 – 4% and stretching vibrations at 1283 cm^{-1}, 1640 cm^{-1}, and 3260 cm^{-1}, respectively. The findings in this study have shown that over-ripe bananas, which were previously thought of as waste, can be transformed into very valuable commodities, consequently enhancing human health and facilitating environmental safety. Results showed that over-ripe banana wort is a good raw material appropriate for fermentation-based ethanol production and vinegar manufacturing using ethanol.

Keywords: Vinegar, *Musa spp, Acetobacter aceti, Saccharomyces cerevisiae,* Over-ripped bananas.

INTRODUCTION

Global population growth results in a strong demand for food and the food industry. Chemically generated preservatives which are linked to carcinogenic contaminants are used to preserve the majority of produced foods (Olga *et al.*, 2021). Everyday fruits that were over-ripe before consumption are thrown away as garbage, this prompted the creation of secure biosynthesized preservatives like vinegar among other considerations. According to Sossou *et al.* (2009), vinegar is an aqueous solution of acetic acid (4 to 8% acetic acid by volume), which may or may not include flavor. It has long been used as a cleaning agent, flavoring agent, preservative for food, and an ingredient in marinades and salad dressings (Singh, 2020). The expected value of the vinegar market is $2.7 billion US up from $2.3 billion by 2028 (Imarc, 2021).

Vinegar works effectively to preserve food and a curing drink (Kulkarni, 2015). According to Marcus (2019), the flavour of vinegar generally gets deeper as it ages. The main flavour of vinegar comes from acetic acid, an antibacterial component (Marcus, 2019). It is the end result of a mixed fermentation process including acetic acid bacterium (*Acetobacter aceti*) and

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Beaker's yeast (*Saccharomyces cerevisiae*) (Ezemba *et al*., 2021). As stated by Frazier and Westhoff (2004), the manufacturing of commercial vinegar frequently calls for a dual process of fermentation employing *Saccharomyces cerevisiae* and *Acetobacter aceti*, respectively, and the fermentation involves both carbohydrates and ethanol. Because different substrates are employed, the microbial fermentation used to make vinegar has the additional benefit of having a cheap production cost (Sinclair, 1998). These include organic food products that can ferment into alcoholic beverages like beer and wine, such as apples, pears, grapes, honey, syrups, cereals, and hydrolyzed starches. The result of this work will review if *Acetobacter aceti* isolated from over-ripped bananas could be used in vinegar production. The purpose of using over-ripped bananas for this production is a way of recycling over-ripped bananas considered wastes into useful substrates and enhancing environmental cleanliness and safety.

MATERIALS AND METHODS

Collection of banana Samples and Processing

Ten (10) over-ripped bananas were bought from a fruit vendor at Dutse Ultra-Modern market, Jigawa State, and taken directly to Federal University Dutse (FUD)'s microbiology laboratory for further analysis.

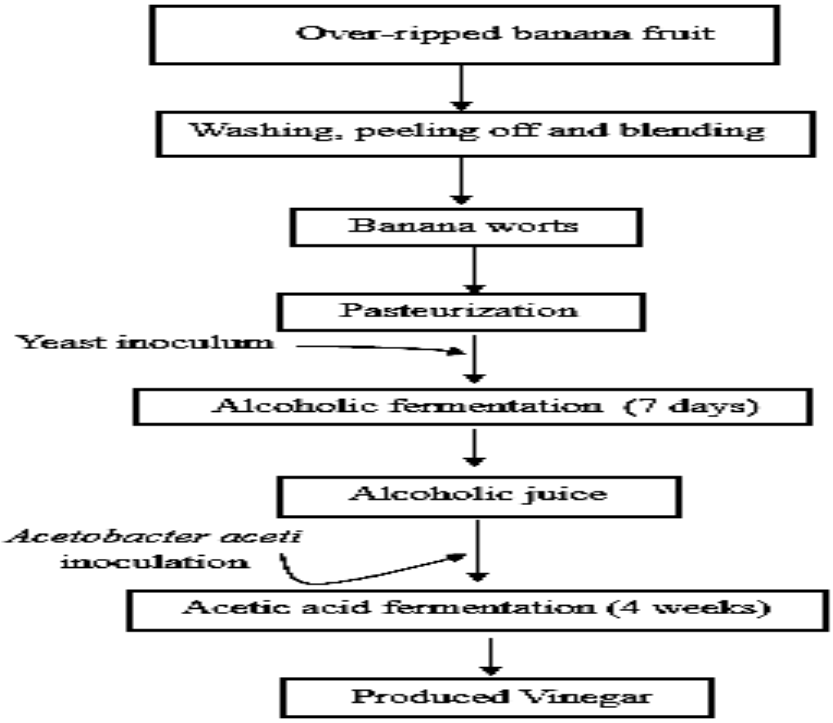

Figure 1: Flow chart showing procedures involved in vinegar production using over-ripped bananas, (Ameyapoh, *et al*. 2010; Adebayo-Oyetoro *et al*. 2017).

Extraction of Soluble Component of the Banana Worts

The over-ripped bananas were selected and then washed using distilled water. The washed bananas were peeled and sliced with a sterilized knife, blended, and filtered to remove rough fibers using a muslin sieve. The extracted soluble component is known as ''wort'' or ''must'' (Lynch, 2016). The worts were boiled for about 30 minutes and occasionally mixed with a sterilized wooden spoon. The boiled wort was allowed to cool and stored in the refrigerator for use.

Enumeration and Isolation of *Acetobacter aceti* from unboil Extracted Worts.
A sample (50 mL) from the prepared worts was collected aseptically, mixed with 50 mL of 0.1 peptone water, and vigorously shaken to homogenate. 0.1 mL of the homogenate sample was diluted serially (Simpson, *et al* 2008). 0.1 mL of the serially diluted sample was spread (inoculated) onto plates of yeast extract agar containing glucose (2.0%), calcium carbonate (2.0%), and distilled water. These plates were incubated for 48 hours at 30 °C, after which the counts for each plate were counted and then expressed as colony forming unit per milliliter of the sample (CFU/mL). Colonies from these plates were sub-cultured separately on yeast extract agar containing 2% ethanol incorporated with congo red (0.5 g) and bromocresol green (0.5 g) indicator at pH 5.5 with cycloheximide in a slant agar (agar slope tube). These tubes were incubated at 30 °C and from colonies in the tubes *Acetobacter aceti* were isolated. *Acetobacter aceti* was identified through Gram staining and biochemical characterization tests ranging from sugar fermentation (fructose, glucose, and sucrose), motility, indole, methyl red, citrate, to Voges-proskauer. These biochemical test were carried out using the procedures reported by Gumel *et al.* (2019) and identified according to Holt *et al.* (1994).

Alcoholic Fermentation
Into a clean, wide-mouthed brewing vessel, the worts were carefully siphoned. 3 g of yeast (*Saccharomyces cereveriae*) were added to the worts in the brewing vessel to produce ethanol from the worts. For seven days, the mixture was allowed to ferment at room temperature. To remove extra yeast and prevent additional fermentation, the worts were filtered using a clean, sterilized muslin cloth at the completion of the fermentation process. For the purpose of fermenting acetic acid, the worts were kept.

Acetic Acid Fermentation
Acetic acid fermentation was carried out using the strain of *Acetobacter aceti*. The inocula of acetic acid bacterium (*Acetobacter aceti)* were added into the alcoholic worts using a wire loop, covered with clean muslin cloth held around the vessel mouth wind round with rubber band to prevent contamination by insects or dust, but allowing air to flow in and out. The mixture was then stored out of direct sunlight and by gently swirling the mixture inside the brewing vessel, once each day, for the first week, and then allowed to ferment at 30 °C for 4 weeks, while acidity was checked every week. At the end of the fermentation, a thick film covering the fermented worts (vinegar) was carefully removed to avoid contamination and the vinegar was filtered afterward.

Physico-chemical Analyses
Determination of the produced vinegar pH
A pH meter (JENWAY) was used in measuring the pH of the fermenting vinegar every week. The electrode was dipped into the fermenting vinegar sample and the values were read and recorded.

Acetic acid assay
Acetic acid levels were measured every seven days after inoculation with *Acetobacter aceti*. In a 250 mL conical flask, 5 mL of vinegar was mixed with 20 mL of distilled water and five (5) drops of phenolphthalein. The mixture was titrated against 0.5 N sodium hydroxide until a pale pink colour appeared in the flask. The amount of sodium hydroxide consumed during the titration was calculated, and the percentage of acetic acid in the vinegar was calculated using stoichiometry (AOAC, 2000) and the formula below:

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

% Acetic acid = Mass of Acetic acid/Mass of Vineger x 100

Fourier transform Infrared assay

The confirmatory test of the produced vinegar was done using Fourier Transform Infrared Spectroscopy (FTIR) conducted on Agilent FTIR-ATR machine at 32 scan rate, wavelength range of 650-4000 cm^{-1}.

RESULTS

Isolation of the Bacterial Inocula

Vinegar production using *Acetobacter aceti*, isolated from over-ripped bananas was carried out. Out of 10 over-ripped bananas analyzed, the bacteria mean count was 0.53 x 10^2 (Table 1).

Table 1: Viable Count of the Bacterial Isolates from the Over-ripped banana fruits.

Sample	Viable counts (x 10^2 CFU/mL)
1	0.45
2	0.43
3	0.52
4	0.63
5	0.45
6	0.50
7	0.49
8	0.58
9	0.60
10	0.64
Mean	**0.53**

The isolate was subjected to morphological test and its colonies were found to appear yellow, flat with negative Gram's reaction (Table 2).

Table 2: Cultural Characteristics and Gram reaction of *Acetobacter* Isolated from Over - ripped bananas

Cultural characteristics after 2 days incubation	Gram's reaction	Presumptive identification	Morphology	Microscopy
Yellow colored and flat small colony	Gram negative	*Acetobacter aceti* sp.	Convex	Rod-like

The isolated bacterium was subjected to biochemical tests as confirmatory tests and was observed to be oxidase negative and indole negative, catalase positive, motility positive, and actively ferment sugars (Table 3).

Table 3: Biochemical Tests for Identification of *Acetobacter aceti* Isolated from Over-ripped Banana fruit.

Biochemical tests	Reactions
Indole	-
Catalase	+
Oxidase	-
Motility	+
Methyl red	-
Fructose	+
Glucose	+
Lactose	+
Sucrose	+

The Acetic Acid Content and pH

The pH and total acidity were measured every week, the pH ranged between 4.4 and 3.8 and the acetic acid content ranged from 1.4% to 4% is presented in (Table 3).

Table 3: Acetic acid content and pH level of the vinegar during fermentation

Week	pH	Acidity (%)
1	4.4	1.4
2	4.2	2.1
3	3.9	3
4	3.8	4

FTIR Analysis

In the FTIR spectra (Figure 2), the stretching vibration at 1283 cm^{-1} was assigned to carbon–carbon single bond (C-C), the stretching vibration at 1640 cm^{-1} was assigned to carbon-oxygen double bond (C=O), and the stretching vibration at 3260 cm^{-1} was assigned to carbon-hydrogen single bond (C-H) and oxygen-hydrogen single bond (O-H).

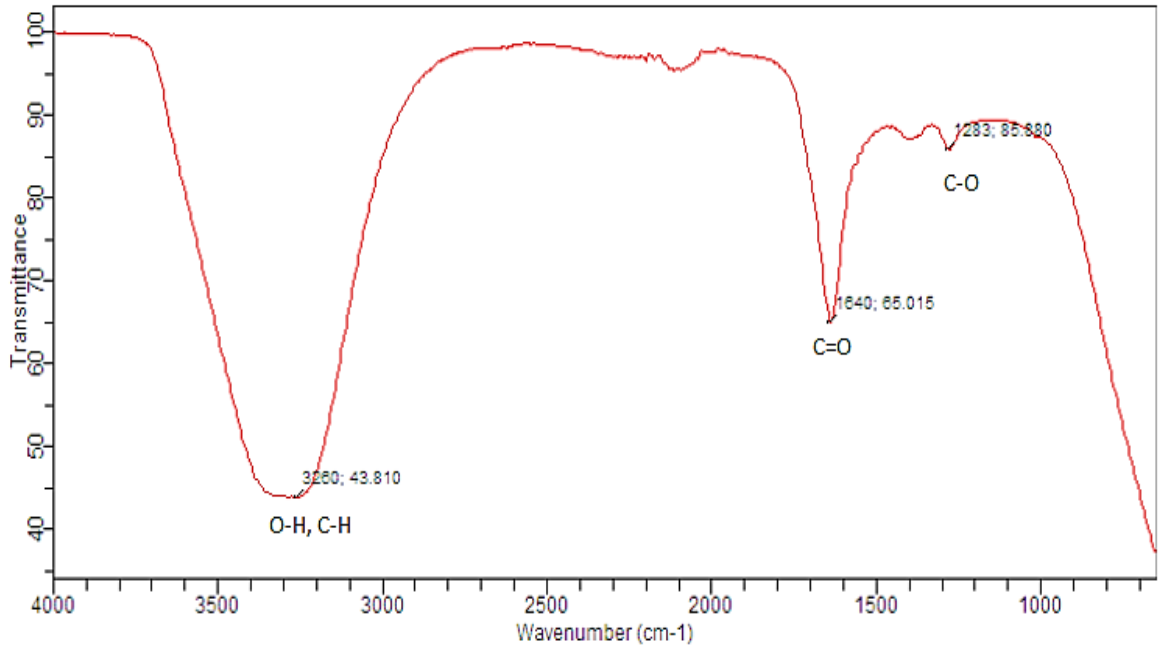

Figure 2: FTIR analysis of vinegar obtained from the fermentation.

DISCUSSION

Hommel and Ahnert (1999) reported that *Acetobacter aceti* can be isolated from various fruits such as rotten (over-ripped) bananas. The results generated in this study has indicated that A*cetobacter aceti* has the ability to oxidize significant quantities of ethanol under the acidic condition created by the presence of acetic acid. Our results agreed with the work of Raji *et al.* (2012); Hickey and Vaughn (1954). The vinegar produced was done using sustainable resources (over-ripped bananas). The bacterium was characterized on their basic of morphology and biochemical characteristics and identified with the scheme of Holt *et al.* (1994). The characteristics recorded in this study indicate that the bacterial isolates might be *Acetobacter aceti*, which is a peritrichous flagella-motile gram-negative bacterium. This agrees with the microscopic analysis of the isolate, which is shown in Table 2.

Further confirmatory tests were observed following biochemical characterization tests that we conducted. The pure culture produced bubbling in the presence of hydrogen peroxide which is an indication of a positive test is shown in Table 3. The results obtained are similar to the findings of Ashecraft *et al.* (2001) who observed that *Acetobacter aceti* has the ability to produce catalase but not oxidase as it was obtained in this study.

As shown in Table 4, the fourth week's acetic acid assay results indicated 4% acidity. The outcomes met the acetic concentration in vinegar sold in retail stores standard (4%–8%), which was established by the Nigerian Standards Organization.

In FTIR test of the vinegar produced, there were different stretching vibrations which illustrated the presence of C-H, O-H, C-C, and a C=O bond indicating the presence of a carboxylic group as depicted in Figure 2. The results obtained are similar to that of Kadiroğlu, (2018); Bryan and Aihui (2015). This study has shown that over-ripped bananas are potential raw materials for the isolation of *Acetobacter aceti,* which could also be used for the production of acetic acid, vinegar, and various useful materials. In this study, the isolation of *Acetobacter aceti* from over-ripped bananas considered as waste was transformed into a high value-added commodity, thereby facilitating environmental safety by reducing the amount of dirt/trash littering around the environment that causes an environmental hazard. The use of this bacterium for such products will be a means of converting over-ripped fruits into useful substrates for the industrial production of vinegar. Economic losses as well as the negative effect on the environment will be drastically reduced if these supposedly tagged waste items are appropriately put into use. The acetic acid content of the produced vinegar conformed to the standard specified by the Standard Organization of Nigeria (SON) for the acetic content of retailed vinegar (4% -8%).

CONCLUSION

Lowering the amount of dirt and rubbish that is left lying around the environment and poses an environmental threat. In this study, the isolation of *Acetobacter aceti* from over-ripe bananas that were deemed waste were transformed into a high value-added commodity, aiding environmental safety in this study. Utilizing this bacterium for such items will allow overripe fruits to be transformed into valuable substrates for the commercial production of vinegar. The vinegar that was made complied with the Standard Organization of Nigerian (SON) requirements that retailed vinegar has.

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