Production of Vinegar from Oil-palm Wine Using *Acetobacter Aceti* Isolated from Rotten Banana Fruits

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Abstract Vinegar production using *Acetobacter aceti* isolated from ten rotten banana fruits collected from different vendors in Eke-Awka Market in Awka, Nigeria was carried out using cultural techniques, with glucose yeast calcium carbonate-ethanol agar as the growth medium. The mean viable count of the bacterial isolates was 0.72×10^2 cfu/g. The isolates were characterized on the basis of their morphological and biochemical characteristics and identified as *Acetobacter aceti* and *Acetobacter orleanensis* with mean counts of 0.53×10^2 cfu/g and 0.19×10^2 cfu/g respectively. Freshly-tapped oil-palm wine was allowed to ferment for seven days at 30°C. The alcohol content was 10.0% while the pH value was 4.6 on the seventh day of the fermentation. The fermented palm wine was further fermented with the *Acetobacter aceti* for four weeks producing vinegar containing 7.1% acetic acid with a pH value of 3.5. The *Acetobacter aceti* grew well in high concentration of alcohol indicating that it is suitable for large scale vinegar production. In addition, the rotten banana fruits regarded as wastes were converted into a useful raw material for the isolation of *Acetobacter aceti* needed for the production of acetic acid and vinegar.

Keywords Vinegar, Palm Wine, *Acetobacter Aceti*, Rotten Banana, *Acetobacter Orleanensis* Nigeria

1. Introduction

Vinegar, which is a good food preservative and a curing drink is the product of mixed fermentation by yeast followed by acetic acid bacteria [1]. It is a food additive that contains essential nutrients such as amino acids and is one of the oldest products of fermentation used by man [2]. It is a 4% acetic acid solution synthesized from sugary substances through alcoholic fermentation [3]. It is the acetic acid produced by the fermentation of alcohol which gives the characteristic flavor and aroma to vinegar.

The world consumption of vinegar was estimated to be

worth almost two hundred and twenty million dollars [4]. However, levels of African vinegar production are difficult to estimate because of the black markets existing in most African countries and until recent years, most of the vinegar used was obtained mainly through the dilution of acetic acid of petrochemical origin.

In many African countries, the production of vinegar is being regulated by the government as new laws come into force [5]. These laws and regulation emphasize that vinegar destined for human use should be produced in a safe and biotechnological way through a double fermentation of agricultural products.

The raw material for vinegar production varies with the various types of vinegar [6] and from one locality to the other, thus, while wine made from grape is common in continental Europe and other growing countries, coconut water vinegar is common in Africa. The basic requirement for vinegar production is a raw material that will undergo an alcoholic fermentation such as apples, pears, grapes, honey, syrups, cereals, hydrolyzed starches, beer and wine [7].

Alcoholic fermentation of carbohydrate is the first step in the production of vinegar and it takes place under anaerobic condition. This step in which sugar is fermented to alcohol is completed by the action of yeast species. Kadere et al [8] highlighted acetic acid fermentation as the second stage in the production of vinegar and it is an aerobic process. This is the step in which alcohol is oxidized to acetic acid by the action of acetic acid bacteria such as *Acetobacter aceti* and *Acetobacter schulzenbachi* [9].

Acetobacter strains are the major acetic acid bacteria that are involved in vinegar production industrially [10]. *Acetobacter aceti* living in certain fruits and flowers are reported in beer and wine spoilage as well as fruit decay [11]. *Acetobacter aceti* is a gram-negative bacterium which is motile by peritrichous flagella, obligately aerobic, possessing only the ability for respiratory metabolism with no fermentation ability and does not form endospores [12]. This bacterium is ubiquitous in the environment, existing in soil, water, flowers, fruits on honey bees, in palm trees, palm wine and in essence wherever sugar fermentation is

occurring.

Acetobacter aceti produces acetic acid from ethanol in alcoholic niches in the environment. Acetate and lactate are oxidized to carbondioxide and water by the organism. The optimal temperature for growth of the bacterium is between 25°C and 30°C and the pH optimum between 5.4 and 6.3. *Acetobacter aceti* is a common contaminant in all industrial fermentation facilities. It is not a pathogen of humans, animals or plants. The potentials risks to human health or the environment associated with the use of this bacterium in fermentation facilities are low.

Acetobacter aceti can be isolated from various substrates such as rotten banana fruits which easily undergo spoilage and are usually discarded as wastes. The aim of this study is therefore to produce vinegar from palm wine using *Acetobacter aceti* isolated from rotten banana fruits. The result of this work will reveal if *Acetobacter aceti* isolated from rotten banana fruits could be used in producing vinegar. The use of this bacterium from the fruit for such production will be a means of converting rotten banana fruits to useful substrates for the industrial production of vinegar. Economic losses as well as the negative effect on the environment will also be drastically reduced.

2. Materials and Methods

2.1. Samples Collection and Processing

Ten samples of rotten banana fruits were obtained from different vendors at Eke-Awka Market, in Anambra State of Nigeria. One gram of each sample was homogenized in sterile distilled water and serially-diluted using a ten-fold dilution technique.

2.2. Isolation of Bacteria

0.1ml of each serially-diluted sample (10^2) was spread-plated on a plate of Glucose Yeast calcium carbonate-ethanol agar (GYC-EA) containing the antifungal agent ketoconazole at a concentration of 0.05mg/ml and 3% ethanol. The composition of the medium was glucose (10%), yeast extract (1.0%), calcium carbonate (2.0%), agar (1.5%) and distilled water (1 litre). The plates were incubated in an inverted position at 30°C for 24 hours, after which the colonies that developed were counted and sub-cultured by streaking on nutrient agar plates to obtain pure cultures. The pure cultures were stored on GYC-EA slants and were used in characterizing and identifying the bacteria.

2.3. Characterization and Identification of the Bacterial Isolates

The strains were characterized and identified using colony morphological characteristics and biochemical tests. Gram staining, sugar (glucose, fructose, sucrose) fermentation,

motility, methyl red, voges proskauer, indole, oxidase, citrate utilization, nitrate reduction, urease, spore and catalase tests were carried out as done by Onuorah et al [13]. They were identified according to Holt et al [14].

2.4. Production of Vinegar

The Orleans method [15] otherwise known as the slow method was adopted in the production of vinegar.

2.4.1. Alcoholic Fermentation

One litre of freshly-tapped palm wine was purchased from a tapper at Amaokwe Udi in Udi Local Government Area of Enugu State and left to ferment at 30°C for seven days to convert the sugars to ethanol by the action of the yeasts present in the palm wine. The palm was decanted through a sterilized sac cloth at the end of the seven days fermentation to remove excess yeast and stop further fermentation. The decanted palm wine (must) was thereafter boiled for one hour, allowed to cool and introduced into a sterile bottle. The alcohol content and pH of the fermenting palm wine were monitored during the seven days of fermentation.

2.4.2. Determination of pH of the Fermenting Palm Wine and Vinegar

A pH meter (JENWAY) was used to measure the pH of the palm wine and vinegar during the fermentation. The meter was first standardized with buffers 4.0 and 7.0, after which its electrode was dipped into the samples and the pH values were read and recorded.

2.4.3. Determination of the Alcohol Content of the Fermenting Palm Wine

An alcohol meter was used to measure the alcohol content of the palm wine during fermentation. The meter was dipped into the fermenting palm wine and the alcohol content was read and recorded.

2.4.4. Acetic Acid Fermentation

One loopful of the isolated *Acetobacter aceti* was inoculated into the sterile bottle containing 250ml of the must. The bottle was thereafter covered with a sac cloth to prevent the entry of insects. The must was allowed to ferment for four weeks at 30°C. At the end of the fermentation period, a thick film known as mother of vinegar had covered the surface of the must and was carefully scooped out to avoid contamination. The vinegar was thereafter filtered.

2.4.5. Acetic Acid Assay of the Vinegar

The assay of acetic acid was carried out every seven days after inoculation with *Acetobacter aceti*. 5mls of the vinegar were added to 20ml of distilled water in a 250ml conical flask and mixed with 5 drops of phenolphthalein. The mixture was titrated against 0.5N sodium hydroxide till the appearance of pale pink colour in the flask. The volume of

sodium hydroxide consumed during the titration was measured and the percentage of acetic acid in the vinegar was calculated using the formular

$$\% \text{ Acetic acid} = \frac{\text{Mass of Acetic Acid}}{\text{Mass of Vinegar}} \times 100$$

3. Results

The viable count of the bacteria isolated from the rotten banana fruits are shown in Table 1. The viable counts ranged between 0.63×10^2 cfu/g and 0.83×10^2 cfu/g.

Table 1. Viable Count Of The Bacterial Isolates From The Rotten Banana fruits

Samples	Viable Counts (x 10^2 cfu/g)
1	0.63
2	0.69
3	0.72
4	0.65
5	0.80
6	0.74
7	0.68
8	0.83
9	0.76
10	0.70
Mean	0.72

The viable counts of the *Acetobacter aceti* and *Acetobacter orleanensis* from the rotten banana fruits are shown in Table 2. The counts were between 0.47×10^2 and 0.63×10^2 cfu/g for *Acetobacter aceti* and between 0.15×10^2 cfu/g and 0.25×10^2 cfu/g for *Acetobacter orleanensis*.

Table 2. Viable Counts Of The *Acetobacter aceti* and *Acetobacter orleanensis* from the Rotten Banana Fruits (x 10^2 cfu/g)

Samples	<i>Acetobacter aceti</i>	<i>Acetobacter orleanensis</i>
1	0.47	0.16
2	0.48	0.21
3	0.54	0.18
4	0.50	0.15
5	0.63	0.17
6	0.49	0.25
7	0.53	0.15
8	0.60	0.23
9	0.51	0.25
10	0.55	0.25
Mean	0.53	0.19

The morphological and biochemical characteristics of the *Acetobacter species* from the rotten banana fruits are

presented in Table 3. *Acetobacter aceti* were rod shaped, opaque, convex in elevation, smooth-surfaced and arranged in clusters, gram-negative, indole negative, spore negative, motile, catalase positive, did not utilize citrate, methyl red positive, voges proskaeur positive, nitrate reduction positive, oxidase negative, urease negative, fermented glucose, fructose and sucrose and produced clear zones that became cloudy while growing on glucose yeast calcium carbonate-ethanol medium while the *Acetobacter orleanensis* were rod-shaped, arranged in clusters, opaque, convex in elevation, smooth-surfaced, gram-negative, indole negative, motile, catalase positive, citrate negative, methyl red positive, voges proskaeur positive, nitrate reduction positive, oxidase negative, urease negative, fermented glucose, did not ferment fructose and sucrose and produced clear zones that did not become cloudy in the centre while growing on glucose yeast calcium carbonate-ethanol medium.

Table 3. Morphological and Biochemical Characteristics of the *Bacterial Isolates* From The Rotten Banana Fruits

Characteristics	Results	
Shape	Rod	Rod
Arrangement	Clusters	Clusters
Opacity	Opaque	Opaque
Elevation	Convex	Convex
Surface	smooth	smooth
Gram reaction	Negative	Negative
Indole	Negative	Negative
Motility	Positive	Positive
Catalase	Positive	Positive
Spore	Negative	Negative
Citrate utilization	Negative	Negative
Methyl red	Positive	Positive
Voges proskaeur	Positive	Positive
Nitrate reduction	Positive	Positive
Oxidase	Negative	Negative
Urease	Negative	Negative
Glucose fermentation	Positive	Positive
Sucrose fermentation	Positive	Negative
Fructose fermentation	Positive	Negative
Growth on Glucose yeast calcium carbonate-ethanol medium	Produced clear zone that became cloudy in the centre	Produced clear zone that did not become cloudy in the centre
Identity	<i>Acetobacter aceti</i>	<i>Acetobacter orleanensis</i>

The alcohol and pH levels of the fermenting palm wine are shown in Table 4. The alcohol levels were between 3.0% and 10.0% while the pH levels were between 4.6 and 6.9.

Table 4. Alcohol and pH Levels of the Fermenting Palm Wine

Days of Fermentation	Alcohol Level (%)	pH Level
1	3.0	6.9
2	3.9	6.7
3	4.3	6.1
4	6.2	5.8
5	7.0	5.4
6	8.5	4.9
7	10.0	4.6

The acetic acid content and pH levels of the vinegar during fermentation are presented in Table 5. The acetic acid content was between 3.8% and 7.1% while the pH ranged between 3.5 and 4.4.

Table 5. Acetic Acid Content and pH Levels of the Vinegar during Fermentation

Weeks of Fermentation	Acetic Acid Content (%)	pH Level
1	3.8	4.4
2	4.5	4.2
3	5.8	3.9
4	7.1	3.5

4. Discussion

The mean count of the bacterial isolates from the rotten banana fruits was 0.72×10^2 cfu/g (Table 1) while the mean counts of the *Acetobacter aceti* and *Acetobacter orleanensis* were 0.53×10^2 cfu/g and 0.19×10^2 cfu/g respectively (Table 2). This result indicated that *Acetobacter aceti* is the major acetic acid bacterium present in rotten banana fruits.

The bacteria were characterized on the basis of their morphological and biochemical characteristics and identified with the scheme of Holt et al [14]. The characteristics indicated that the isolates were *Acetobacter aceti* and *Acetobacter orleanensis* (Table 3). Hommel et al [11] reported that *Acetobacter aceti* can be isolated from various fruits such as plantain fruits. *Acetobacter aceti* is a gram-negative bacterium which is motile by peritrichous flagella and does not form endospores [12].

The alcohol content of the fermenting palm wine was the lowest on the first day of fermentation (3.0%) but increased progressively as the days of fermentation increased, with the highest value of 10.0% on the seventh day of the fermentation (Table 4). This result indicated that palm wine produces high level of alcohol when allowed to ferment, thus making it a suitable substrate for vinegar production. The highest pH value of 6.9 was recorded on the first day of the fermentation of the palm wine. This value decreased as the days of the fermentation increased, with the lowest value of 4.6 obtained on the seventh day of the fermentation. This result is in agreement with the works of Okafor [16] and Faparusi and Bashir [17] who reported an increase in alcohol content and a decrease in pH level of palm wine during

fermentation. The result also indicated that the low pH favoured alcohol production by the yeasts and inhibited the growth of contaminating microorganisms.

The acetic acid content of the vinegar was lowest (3.8%) during the first week of fermentation of the alcohol with *Acetobacter aceti* but increased to 7.1% during the fourth week of fermentation (Table 5). In addition, the pH decreased progressively from 4.4 during the first week of fermentation of the alcohol to 3.5 during the fourth week of the fermentation. This result indicated that *Acetobacter aceti* has the ability to oxidize significant quantities of ethanol under the acidic conditions created by the presence of acetic acid and agreed with the work of Stanier [18] and Hickey Richard and Vaughn Reese [15].

Palm wine despite being a rich medium with high concentration of hexoses (200-250g/l), high pH (5.17-6.92) and titrable acidity (2-10g/l) as tartaric acid provides a highly selective medium for bacterial growth. In addition, the harsh environment of the fermenting palm wine created by high ethanol concentration, and low pH resulting from the consumption of sugars by yeasts during alcoholic fermentation restricted the growth of other bacterial species [19].

Acetobacter species are better adapted to higher ethanol concentrations and thus tend to colonize fermented palm wine, thereby causing the sour taste [20]. This work is in agreement with the study of Kocher et al [21] on the production of vinegar using *Acetobacter aceti* isolated from fruits in terms of the tolerance of the bacteria to high alcohol content and high production of acetic acid in vinegar.

The acetic acid content of the vinegar produced in this work conformed to the standard specified by the Standards Organization of Nigeria for acetic content of retailed vinegar (4%-8%), thus from the results obtained, rotten banana fruit is a potential substrate for the isolation of *Acetobacter aceti* for the production of acetic acid and wide varieties of vinegar.

5. Conclusions

The result of this work showed that *Acetobacter aceti* can be isolated from rotten banana fruits and they can withstand high alcohol content and low pH. Furthermore, the rotten banana fruits regarded as wastes can be a useful raw material for the isolation of *Acetobacter aceti* needed for the industrial production of acetic acid and vinegar.

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