https://dx.doi.org/10.4314/ijs.v26i1.14

PRODUCTION OF VINEGAR FROM WASTE FRUITS USING *Acetobacter* SPECIES

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(Received: 21st February, 2023; Accepted: 11th April, 2024)

ABSTRACT

Vinegar is an organic substance that contains about 5% acetic acid and is made from the oxidative fermentation of alcohol by acetic acid bacteria. The aim of this study was to produce vinegar from the juices of waste fruits. Proximate and physicochemical analysis was carried out on the juices of orange, sugarcane, tomato, and pineapple using standard techniques. Twenty-one (21) species of acetic acid bacteria were isolated and identified using glucose-yeast-Calciumcarbonate (GYC) agar. The juices were fermented for 7 days using *Saccharomyces cerevisiae*, and subsequently, for 28 days, four alcohol-tolerant *Acetobacter* species were selected and inoculated into the fermenting media. The produced vinegar was sensory evaluated, following a nine-point hedonic scale. The antibacterial and antifungal effects of the vinegar were tested on fresh cabbage at various contact times (0, 5, and 10 min) and concentrations (5, 10, and 15 % v/v). The physicochemical parameters analyzed in fermentation using *S. cerevisiae* showed a decrease in pH, specific gravity, and brix, while the percentage of alcohol content increased. As the fermentation using *Acetobacter* species progressed, pH further decreased, titratable acidity increased in a range of 6.6-7.7%, and specific gravity also decreased while specific gravity increased in Acetic acid fermentation. Sensory evaluation showed a score range of 5.7–6.8 for pungency, 6.8–7.4 for appearance, 6.4–7.9 for aroma, 5.8–7.4 for taste, and 6.9–7.9 for acceptability. The best results for bacterial and fungal loads on cabbage were at a concentration of 15% at 10 min. Drastic reduction of bacterial load was from 2.07×10^5 to 2.7×10^4 CFU/g, while fungal load reduced from 4.9×10^4 to 6.7×10^3 CFU/g. To evaluate significance, an ANOVA was performed ($p<0.05$) and Duncan's test was used for multiple comparisons. Vinegar was successfully tested to be effective as an antimicrobial agent. Future research should look into process optimization, strain improvement, and the adoption of agro-allied wastes for biotechnology.

Keywords: Vinegar, *Acetobacter*, Proximate analysis, Sensory analysis, Antimicrobial.

INTRODUCTION

Vinegar can be described as a condiment made from diverse sugary and starchy substances through alcoholic and acetic fermentation (Tan, 2005). It is the acetic acid produced by the fermentation of alcohol that gives vinegar its characteristic flavor and aroma (Onuorah *et al.*, 2016). It is an organic substance that contains about 5% of acetic acid (Bhat *et al.*, 2014). Although a major percentage of vinegar is composed of acetic acid, vinegar is a dilute form of acetic acid (Ouattara *et al.*, 2019). When fermentation takes place, other organic compounds are produced and added, depending on the substrate used (Okafor, 2007). The major component of vinegar that adds flavor and antimicrobial properties is acetic acid. Purified acetic acid is widely employed in the production of commodity chemicals, namely vinyl acetate, acetic anhydride, cellulose, and acetate (Tumane *et al.*, 2018). Vinegar's antimicrobial properties make it useful in the treatment of fungal infections (Ali *et al.*, 2016). When consumed, it balances high blood sugar levels and treats insulin response levels (Ayoub *et al.*, 2016; Ho *et al.*, 2017b). In the food industry, it serves as an antimicrobial agent, a food additive, and a preservative. Microorganisms that produce acetic acid through oxidation are called acetic acid bacteria (Selvanathan *et al.*, 2020). They are gram-negative, rod-shaped, strict aerobes (Gullo *et al.*, 2014). Previously, acetic acid bacteria were classified into the genera *Acetobacter* and *Gluconobacter*. Presently, there are twelve genera in the family of *Acetobacteriaceae* (Ouattara *et al.*, 2019). The genus *Acetobacter* is commonly preferred for the production of vinegar (Tumane *et al.*, 2018). This study discourages over-dependence on importation, provides an option for the exploitation of existing fruit waste and farm surpluses, and promotes the use of microorganisms in biotechnology over conventional methods of production due to their high purity, selectivity, cost-effectiveness, and environmentally friendly nature. This study focuses on the production of vinegar from fruit wastes using *Acetobacter* species.

MATERIALS AND METHODS

Collection and preparation of Samples

Wastes of pineapple, tomato, orange, banana, watermelon, grape, and sugarcane were aseptically collected into sterile polythene bags from fruit sellers at the sorting out point in Station Market, Kaduna. They were transported to the laboratory of the Department of Microbiology, Kaduna State University (Mansor *et al.*, 2012). They were washed thoroughly with water to remove sand (Tumane *et al.*, 2018). The juices were obtained by using a clean blender or mechanical squeezing and crushing. One (1.0) L of each juice sample was transferred into clean plastic bottles (Ameyapoh *et al.*, 2019). The manufacturer's instructions for preparing potato dextrose agar (PDA) were adopted. Thirty-nine (39) g of dehydrated media (PDA) were measured using a weighing balance. It was dissolved in 800 mL of sterile distilled water in a 1000 mL flask, then swirled. Commercially obtained chloramphenicol was obtained, and 0.1 g was weighed and added to the flask. The mixture was heated on a hot plate until it was clearly dissolved. When it was completely dissolved, it was plugged with cotton wool wrapped in foil paper and sterilized.

Glucose yeast extract calcium carbonate (GYC) agar was prepared by weighing 5.0 g of glucose, 1.0 g of yeast extract, 0.5 g of calcium carbonate, and 2.0 g of agar (Martinez, 2014). Carr media was prepared by measuring 7.5 g of yeast extract and 5.0 g of agar. These were dissolved in 250 mL of sterile distilled water. This mixture was heated to 100 °C to dissolve and sterilized in an autoclave. When it was cool, 5.0 mL of ethanol and a drop of bromophenol blue (100 mg/mL) indicator was added to the medium and vigorously mixed until there was a consistent blue color (Sharafi *et al.*, 2010; Yanti *et al.*, 2017).

Determination of the Proximate Composition of Juices.

One hundred (100) mL of orange, sugarcane, pineapple, and tomato juices were transferred into clean plastic bottles and immediately taken for proximate analysis at the Institute for Agricultural Research at Ahmadu Bello University, Zaria. The percentage moisture content, ash content, fiber content, lipid content, protein content, and carbohydrate content were determined according to the standard procedure of AOAC, 2010.

Determination of Reducing Sugar Concentration in Juices by Dinitrosalicylic Acid (DNSA) Method

Two (2.0) mL of DNSA reagent was added to a 2.0 mL juice sample in a lightly capped test tube. The test tube was covered with a piece of cotton wool wrapped in foil paper to avoid evaporation. The mixture was heated in a water bath at 60 °C for 5 min to develop the red-brown color. After cooling at 25 °C, 1.0 mL of a 40% potassium sodium tartrate (Rochelle salt) solution was added to stabilize the color. This procedure was carried out with glucose as the standard. Absorbance was measured by transferring 4.0 mL of the mixture into a cuvette, which was then placed in a spectrophotometer (SpectrumLab 23A) at 540 nm. Ethanol was initially used as a blank to calibrate the spectrophotometer before taking readings for optical density (O.D.) (Lee *et al.*, 2013; Walia *et al.*, 2013; DE, 2014).

Determination of pH of Juices

A digital pH meter (OHAUS Starter 2100) was used to measure the pH of the juices. The meter was standardized with buffers pH 4.0 and 7.0, after which its electrode was dipped into the juice, and the pH values were read and recorded.

Determination of the Specific Gravity of Juices

A density bottle was washed, oven-dried, cooled, and weighed using a weighing balance. Fifty (50) mL of the juice sample was measured in the empty density bottle and weighed. An equal volume of distilled water was also measured in the density bottle and weighed. The specific gravity (SG) was calculated using the formula from A.O.A.C. (2010). S.G =weight of juice sample ÷ weight of equal volume of water.

Determination of Degree Brix and Alcohol Content of Juices

Brix refers to 1.0 g of dissolved sugar in 100 g of solution. The value of brix was calculated by comparing the measured specific gravity value of the juice or the fermented juice with the specific gravity-brix conversion table obtained from the U.S. Department of Agriculture (1981). Alcohol by volume (ABV) is the percentage of potential

alcohol that was produced by the equivalent gram of sugar available in the juice. The percentage of alcohol was therefore calculated from the mean value of brix by using the formula from Christensen (2019):
Alcohol content (%) = ABV (1.0) minus ABV (n)
Where: ABV (1.0) = initial potential alcohol by volume reading and
ABV (n) = succeeding alcohol by volume reading.

Estimation of the Percentage of Titratable Acidity (%TTA)

Titration was used to determine the acetic acid content of vinegar. A 0.5 N NaOH was freshly prepared. This solution was used as the titrant. Five (5) mL of vinegar was measured using a pipette and transferred into a 250-mL conical flask, which contain 20 mL of distilled water. Two drops of phenolphthalein were added to the flask as an indicator. The flask was placed on a white tile for visibility. Sodium hydroxide was poured into a burette, up to the zero mark. It was held with a clamp, and the flask containing the vinegar was placed below it. The tap of the burette was opened gently to allow the NaOH to pour down into the flask. While this was going on, the flask was gently swirled to mix the reactions. This continued until the colorless solution turned pink, and then the volume of NaOH used was recorded as the end point. This was repeated for all the vinegar samples. To estimate the percentage of acetic acid in vinegar, the endpoint of the titration was recorded and used in the formula below:

$$\%TTA = \frac{N \times Vn \times WW}{Vv \times 1000} \times 100$$ (A.O.A.C, 2010).

N = normality of NaOH (0.5), Vn = volume of NaOH used (mL), MW = molecular weight of acetic acid (60.05 mg), Vv = volume of vinegar sample (mL). 1000 = factor relating mg to grams (mg/g) (1/10 = 100/1000) (Braide *et al.*, 2011; Ezemba *et al.*, 2021).

Isolation of *Saccharomyces cerevisiae* and *Acetobacter* species

Saccharomyces cerevisiae was isolated through the spread plate technique. Ripe grapes were mechanically squeezed out into a sterile beaker. One (1.0 mL) of the juice was pipetted into 9.0 mL of sterile distilled water and swirled. A five-fold serial dilution of each of the grape juices was made. One-tenth of a milliliter (0.1 mL) of each serially diluted juice sample in each of the five test tubes was pipetted on the surface of the solidified PDA and spread with a sterilized bent glass rod. An extra plate, which was not inoculated, was used as a control. All the inoculated petri plates were incubated at 25 °C for 48 h. The isolated colonies were sub cultured and purified by re-streaking on sterile PDA plates. The pure isolates were maintained on PDA slants in the refrigerator at 4 °C (Chatterjee *et al.*, 2011). The aseptic technique was strictly adhered to.

One (1.0) mL of each of the orange, sugarcane, banana, pineapple, watermelon, and tomato juices was transferred into a clean test tube containing 9.0 mL of sterile distilled water. Further dilution was made by transferring 1.0 mL from the first test tube into four more test tubes. From each of the dilutions, 0.1 mL was spread on the surface of GYC agar plates with the use of a sterile bent rod inside a sterile laminar flow condition. This was done in duplicates for each juice and was labeled. An extra plate was used as a control. The inoculated GYC agar plates were incubated at room temperature (25 °C) for 48 h, in an inverted position. Observations were made for the production of a clear ring or halo around the colonies grown on the GYC medium, which indicated the production of acetic acid that dissolved $CaCO_3$ (Mansor *et al.*, 2012). The colonies that appeared singly were picked using a sterile wire loop and then subcultured by the streak plate technique on freshly prepared GYC agar plates to obtain a pure culture. The pure cultures obtained after incubation were stored on slants of GYC agar (Tharine *et al.*, 2015; Ezemba *et al.*, 2021).

Identification of *Saccharomyces cerevisiae* and *Acetobacter* species

Cultural characteristics were observed in distinct colonies of *S. cerevisiae* for growth pattern, elevation, texture, margin, opacity, conidial morphology, and pigmentation. A wet mount method was adopted for the identification of cells, as described by Ezemba (2022).

After incubation of *Acetobacter* species at 25 °C for 48 h, the plates were carefully observed for cultural characteristics such as size, elevation,

shape, opacity, elevation, color, surface texture, and consistency. A careful morphological observation was made of the shape of cells, the arrangement of cells, the color of cells, and the presence of spores (Kowser *et al.*, 2015). A test for bacteria motility was carried out using the hanging drop slide technique, and Gram staining was carried out as described by Ezemba (2022).

Sugar Utilization and Alcohol Tolerance Test for *Saccharomyces cerevisiae* and *Acetobacter* species

The utilization of sugars by *Saccharomyces cerevisiae* and *Acetobacter* species through the process of fermentation was studied. Each of the sugars (glucose, sucrose, fructose, lactose, maltose, mannitol, and xylose) was prepared by dissolving 20 g in 100 mL of sterile distilled water in a beaker (Ezemba, 2022). The test tubes, which contained 10 mL of peptone water and inverted Durham's tubes, were sterilized in an autoclave at 121 °C for 15 min. After cooling, the peptone water in each test tube was reduced to 9.9 mL. A drop of neutral red indicator was added to each test tube, and then 0.1 mL from each of the prepared sugar solutions was added to each test tube. A sterile wire loop was used to pick a colony of each of the cultures and inoculate it into each of the test tubes. An extra test tube that was not inoculated was used as a control. The tubes were incubated for 24 h at 30 °C. An observation was made for the change of color from red to yellow and the presence of gas bubbles trapped in the Durham tube (Kowser *et al.*, 2015; Antia *et al.*, 2018).

A loop full of all the test isolates was inoculated into five test tubes, each containing 10 mL of freshly prepared yeast extract broth (3.0 g yeast extract, 10 g peptone, and 5.0 g NaCl in 1.0 L sterile distilled water), which were supplemented with different concentrations of ethanol (4.0, 8.0, 12, 16, and 20% v/v). An extra uninoculated tube served as a control. The tubes were incubated for 24 h at 30 °C. The tubes were observed for turbidity (Abubakar, 2017). The ability to grow in alcohol is the test that was used to select the acetic acid bacteria that will be used for the production of vinegar. This is because the bacteria will be introduced into a medium that contains alcohol resulting from the alcoholic fermentation of juices. This method was adopted from Tharinee *et al.* (2015).

Biochemical Test for Isolated Acetic Acid Bacteria

The catalase test, oxidase test, and citrate test were carried out using the methods described in the laboratory procedures manual and book of Prescott and Harley (2002), Sagar (2019), and Ezemba (2022).

Identification of *Acetobacter* species on Carr Medium

A loop full of acetic acid bacteria colonies was inoculated in yeast extract broth (3.0 g yeast extract, 10 g peptone, and 5.0 g NaCl in 1 L sterile distilled water) and incubated for 24 h. Carr media was aseptically poured into petri dishes and allowed to cool. Then 0.1 mL of the incubated acetic acid bacteria culture was spread with the use of a sterile glass rod on Carr media. It was incubated at 37 °C, and growth was observed after 24 h. This medium usually shows a yellow ring around the bacteria colony if it is able to produce acetic acid (Diba *et al.*, 2015; Bellakinmath *et al.*, 2017).

Inoculum Preparation and Standardization of Isolates

Isolates P1, T3, O2, and S1 were selected and named AAB1, AAB2, AAB3, and AAB4, respectively. Each of them was transferred into a separate sterile conical flask, which contained 10 mL of freshly prepared nutrient broth medium. The conical flasks were loaded on an orbital shaker for incubation. It was set at a speed of 150 xg at 37 °C for a period 24 h, to build up inoculum.

A 1.0 McFarland standard was prepared. It was prepared by mixing 1.0 mL of 1.175% barium chloride dihydrate ($BaCl_2$+ $2H_2O$) with 9.0 mL of 1% sulfuric acid (H_2SO_4) in a sterile test tube. A 1.0 McFarland standard concentration amounts to $3x10^8$ cells (Scott, 2011; McFarland Standards, 2022). After 24 h of incubation, six sterile test tubes were filled with 9.0 mL of sterile distilled water. One (1.0) mL from each prepared inoculum was taken separately from the conical flask and diluted serially in the six test tubes. The test tube containing the prepared McFarland standard was compared with each of the six test tubes by placing it by the side. This was done in the

presence of sunlight close to a window, and white cardboard paper was used as a background. The test tube containing 10^6 dilutions matched the 1.0 McFarland standard and was selected. One (1.0) mL of 10^6 dilution was placed in the cuvette of the Ultra Violet-Visible Spectrophotometer (SpectrumLab 23A), and absorbance was read at a wavelength of 600nm (OD600). From the 10^6 dilution for each of the isolates, 0.1 mL was inoculated on a freshly prepared PDA in a petri dish. The plates were incubated for 24 h at 37 °C. The number of colonies was counted with a colony counter and recorded (Pfaller *et al.*, 1988). The concentration of *Saccharomyces cerevisiae* was 2.85×10^7 cfu/mL at 0.22 OD, AAB 1 was 2.25×10^7 cfu/mL at 1.21 O.D, AAB 2 was 2.5×10^8 cfu/mL at 0.28, while AAB 3 and AAB 4 had concentrations of 2.71×10^7 at 0.30 and 2.36×10^7 cfu/mL at 0.24, respectively.

Production of Crude Vinegar from Juices

This was carried out in four Bama bottles for each orange, sugarcane, pineapple, and tomato. Four other bama bottles were used as controls (Reddy *et al.*, 2011). One hundred (100) mL of each of the juices were aseptically poured into each of the Bama bottles and covered. One (1.0) mL of the standardized 10^6 inoculum culture of *Saccharomyces cerevisiae* was added to the four juice samples except for the four control bottles (Tumane *et al.*, 2018). All the bottles were incubated at 30 °C for 144 h (Ameyapoh *et al.*, 2010). As the fermentation progressed to 7 days, daily readings of specific gravity, Brix, percentage alcohol, and pH were taken (Aminu *et al.*, 2018).

After the fermentation of juices into alcohol, 1 mL from each of the four 10^6 standard acetic acid bacteria cultures named AAB1, AAB2, AAB3, and AAB4 was inoculated into each of the four fermenting juices: orange, sugarcane, pineapple, and tomato, respectively. The bottles were labeled with the four selected acetic acid bacteria. Cheesecloth was used to cover the surface to an ideal mouth diameter to minimize the loss of volatile components of vinegar, which have a direct effect on flavor and odor development (Beltran-Milan *et al.*, 2016). These were kept for 28 days for the acetic acid bacteria to oxidize the alcohol into acetic acid. From the first day and every seven-day interval, readings were taken for pH, the volume of NaOH used, titratable acidity, and specific gravity. After 28 days, the solid mass layer on the fermentation medium was gently removed (Ouattara *et al.*, 2018).

Separation of crude vinegar from the fermentation medium

After the 28 days of fermentation, the fermented medium was aseptically filtered using a clean filter cloth. The filtrate was used to fill 10 Eppendorf tubes and was centrifuged at 10,000 rpm for 10 min. The supernatant was poured out, and the remaining extract was aseptically poured into clean 20-mL plastic bottles (Tumane *et al.*, 2018; Perumpuli *et al.*, 2022).

Qualitative test for acetic acid

One (1.0) mL of crude vinegar extract was aseptically poured into a sterile test tube, followed by the addition of sodium bicarbonate. There was effervescence due to the formation of carbon dioxide gas, which is the product of the reaction of acetic acid and sodium bicarbonate (Tumane *et al.*, 2018).

Assay of Acetic Acid by Estimation of the Percentage of Acetic Acid in Vinegar

At an interval of seven days, 5.0 mL of the sample was transferred into a 250 mL conical flask that contained 20 mL of sterile distilled water. Five drops of phenolphthalein indicator were added. A burette filled with 0.5N sodium hydroxide was set on a clamp and used for titration until there was a consistent pink color (A.O.A.C., 2010).

Sensory Evaluation

The vinegar was evaluated by a 20-man panel of postgraduate students and staff of Kaduna State University, all of whom received a brief training and orientation on sensory evaluation and the properties of vinegar. Their evaluation was based on a well-structured and modified nine-point hedonic scale adopted from Bayram *et al.* (2020) and Ezemba *et al.* (2021). It consisted of descriptors, namely, color or appearance, fruity aroma, pungency, taste, and acceptability. These descriptors were quantified as: (1.0) like extremely, (2) like v/much, (3) like moderately, (4) like slightly, (5) neither like nor dislike, (6) dislike slightly, (7) dislike moderately, (8) dislike

extremely, and (9) dislike v/much. The evaluations took place in the morning from 10:00 to 11:30 a.m. at room temperature using natural light (Adebayo-Oyetoro *et al.*, 2017).

Test for the Effect of Crude Vinegar on the Microbial Load of Cabbage

Ten (10) g of unwashed cabbage samples were weighed in four sterile polythene bags. The first sample was homogenized using a sterile blender with 90 mL of sterile distilled water. Subsequently, 0.1 mL of the homogenate was serially (100 fold) diluted, and 0.1 mL of 10^{-4} dilution was pipetted onto solid nutrient agar. A sterile glass rod was used to spread the aliquot evenly on the surface of the solid nutrient agar. The inoculated plates were incubated at 25 °C for 24 h for bacterial counts. The second, third, and fourth samples of 10 g of cabbage were washed thoroughly in three concentrations of diluted vinegar. The concentrations were made in three parts: 5 mL of vinegar was diluted in 95 mL of water in a sterile beaker to give a 5% solution; 10 mL of vinegar was also diluted in 90 mL to give a 10% (v/v) solution; and 15 mL of vinegar gave a 15% (v/v) solution. They were washed for 5 and 10 min. Then they were homogenized in 90 mL of sterile distilled water. From the homogenate, 0.1 mL was taken, and then 0.1 mL of its dilution (10^{-4}) was inoculated on nutrient agar. The plates were incubated at 25°C for 24 h for bacterial counts. The fungal count was also carried out using an aliquot of 1.0 mL from 10^{-4} dilution which was inoculated on potato dextrose agar and incubated at 25 °C for 48 h. After incubation, colonies were counted using a colony counter, and colony-forming units per gram were calculated and recorded. The procedure was adopted from Kabir *et al.* (2022), Kyari (2022), and Traore *et al.* (2022).

Statistical Analysis

The results were expressed as the mean ± standard deviation (SD). Data was analyzed using one-way analysis of variance (ANOVA) to determine the statistical significance within an alpha value of 0.05. A post-hoc test was carried out using Duncan's multiple comparison, using Statistical Package for Social Science (SPSS) version 25. Microsoft Excel 2016 was used to plot charts.

RESULTS

Proximate composition is presented in Table 1, for tomato, orange, pineapple and sugarcane. In terms of the highest for all the parameters, Sugarcane had 82.2 ± 0.286 moisture content, pineapple 0.8±0.025 ash content, tomato juice 13.3 ± 0.281 lipid content, orange juice 2.8 ± 0.131 protein content, no fibre content and carbohydrate content 9.7 ± 0.349 in tomato juice.

Table 1: Proximate Composition of Juices

Juice	Parameters (%)					
	Moisture	Ash	Lipid	Protein	Fibre	Carbohydrate
Tomato	75.4±0.126^{a}	0.6±0.025^{a}	13.3±0.281^{a}	1.2±0.070^{a}	0.0±0.000	9.7±0.349^{a}
Orange	77.4±0.306^{b}	0.6±0.010^{b}	11.7±0.334^{b}	2.8±0.131^{a}	0.0±0.000	6.9±0.589^{a}
Pineapple	80.8±0.305^{c}	0.8±0.025^{c}	9.6±0.533^{c}	1.9±0.020^{ab}	0.0±0.000	6.9±0.858^{b}
Sugarcane	82.2±0.286^{d}	0.4±0.025^{d}	7.3±0.119^{d}	1.1±0.032^{b}	0.0±0.000	8.9±0.322^{b}

Values are Means of triplicate ± Standard Deviation,
Values with different superscript within the same column have significant difference ($p < 0.05$)

Shown on figure 1, are the readings for reducing sugar concentrations in juices and optical densities were measured at wavelength 540 nm. The highest to the lowest was 1.6 for sugarcane juice, 1.53 for pineapple juice, 1.5 for orange juice, and 1.2 for tomato juice.

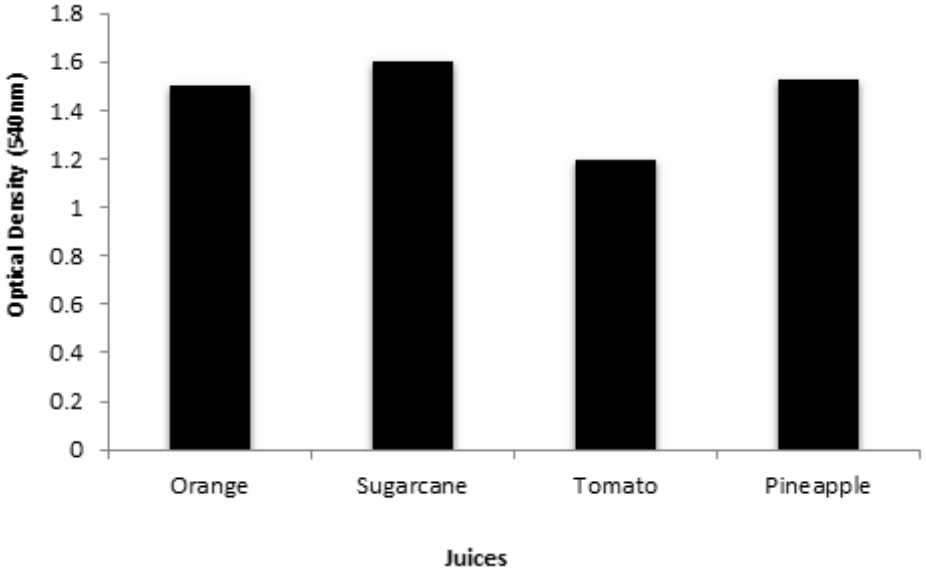

Figure 1: Reducing Sugar Concentration of the Juices

Table 2 shows observation of yeast colonies. They looked small in size, and round in shape, and some were raised while some had a flat elevation. They were creamy in color, and moist in texture. They have no filaments or sporulation. Microscopic observation shows the structure and shape of the yeast cells. They were single cells, oval or spherical, and appeared with buds.

Table 2: Macroscopic and Microscopic Characteristics of *Saccharomyces cerevisiae*

Macroscopic Characteristics	Microscopic Characteristics	Probable fungi
Small round colonies, flat and raised elevation, creamy in colour, moist with no filaments and no sporulation.	Hyphae are absent. Single cells, oval or spherical, appear with buds which are pseudo-hyphae.	*Saccharomyces cerevisiae*

A total of 22 isolates were tested for sugar utilization and alcohol tolerance as shown in Table 3. Yeast was isolated from grape juice. The isolates are represented by the initials of their juice sources; pineapple, tomato, orange, banana, sugarcane and watermelon. All were able to utilize glucose, sucrose, and fructose. All isolates were able to utilize xylose except T2 and S4. All isolates were able to utilize Mannitol except P3, P4, T2, O1, O2 and B3. None of the isolates was able to utilize lactose and maltose. In the test for *Saccharomyces cerevisiae* glucose, sucrose, fructose and maltose produced acid and gas, while lactose, mannitol and xylose did not produce acid or gas. *Saccharomyces cerevisiae* at 0%, 4%, 8%, showed intensive growth, which was observed by turbidity. At 12% and 16 % there was moderate growth, and at 20% concentration of alcohol, there was low growth. Acetic acid bacteria show that at 4% there was intensive growth of all isolates. At 6 % P1, P2, T3, O1, O3 and S1 shows intensive growth, T1, T2, O2, O4, B1, B2, B3, S2 and S3 showed moderate growth, P3, P4, S4, W1, W2 and W3 showed low growth. At 8%, P1, T3, O3 and S1 showed intensive growth, P2 and O1 showed moderate growth, P3, P4, T2, O2, O4, B1, B2, B3, S2, S3, S4 and W3 showed low growth, while T1, W1 and W2 showed no growth. At 10% there was no intensive growth, P1, T3, O2 and S1 showed no growth.

Table 3: Sugar Utilization and Alcohol Tolerance of the Isolates

Isolate	Sugar							Alcohol (%)							
								0	4	6	8	10	12	16	20
	Glucose	Sucrose	Fructose	Lactose	Maltose	Mannitol	Xylose								
SC	AG	AG	AG	-	AG	-	-	+++	+++	*	+++	*	++	++	+
P1	AG	AG	AG	-	-	AG	AG	+++	+++	+++	+++	++	*	*	*
P2	AG	AG	AG	-	-	AG	AG	+++	+++	+++	++	-	*	*	*
P3	AG	AG	AG	-	-	-	AG	+++	+++	+	+	-	*	*	*
P4	AG	AG	AG	-	-	-	AG	+++	+++	+	+	-	*	*	*
T1	AG	AG	AG	-	-	AG	AG	+++	+++	++	-	-	*	*	*
T2	AG	AG	AG	-	-	-	-	+++	+++	++	+	-	*	*	*
T3	AG	AG	AG	-	-	AG	AG	+++	+++	+++	+++	++	*	*	*
O1	AG	AG	AG	-	-	-	AG	+++	+++	+++	++	-	*	*	*
O2	AG	AG	AG	-	-	-	AG	+++	+++	++	++	++	*	*	*
O3	AG	AG	AG	-	-	AG	AG	+++	+++	+++	++	-	*	*	*
O4	AG	AG	AG	-	-	AG	AG	+++	+++	++	+	-	*	*	*
B1	AG	AG	AG	-	-	AG	AG	+++	+++	++	+	-	*	*	*
B2	AG	AG	AG	-	-	AG	AG	+++	+++	++	+	-	*	*	*
B3	AG	AG	AG	-	-	-	AG	+++	+++	++	+	-	*	*	*
S1	AG	AG	AG	-	-	AG	AG	+++	+++	+++	+++	++	*	*	*
S2	AG	AG	AG	-	-	AG	AG	+++	+++	++	+	-	*	*	*
S3	AG	AG	AG	-	-	AG	AG	+++	+++	++	+	-	*	*	*
S4	AG	AG	AG	-	-	AG	-	+++	+++	+	+	-	*	*	*
W1	AG	AG	AG	-	-	AG	AG	+++	+++	+	-	-	*	*	*
W2	AG	AG	AG	-	-	AG	AG	+++	+++	+	-	-	*	*	*
W3	AG	AG	AG	-	-	AG	AG	+++	+++	+	+	-	*	*	*

Key: A=Acid, G=gas, - = no reaction, + = low growth, ++ = moderate growth, +++ = intensive growth *= nill, SC- *Saccharomyces cerevisiae*, P- Pineapple, T-Tomato, O-Orange, B-Banana, S-Sugarcane juice, W-Watermelon, + = positive, -= negative, S-P-C =single, pair, chain, <1 = less than

Table 4: Cultural and Morphological Characteristics of Acetic Acid Bacteria

A total of 21 distinct bacteria isolates were identified as *Bacillus* species based on their morphological characteristics. The cells are rod-shaped, appear singly or arranged in chains or pairs, pink in color, gram-negative, motile, and without spores. They are all less than 1 millimeter in size. They have circular/irregular shapes, white/creamy colors, opaque opacity, slimy consistency, raised elevation, and a moist/smooth surface.

Table 4: Cultural and Morphological Characteristics of Acetic Acid Bacteria

	Cultural							Morphological					
Isolate	**Shape**	**Size**	**Colour**	**Opacity**	**Elevation**	**Surface**	**CT**	**Shape**	**Arrangement**	**Colour**	**GS**	**Motility**	**Spore**
P1	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
P2	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
P3	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
P4	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
T1	Irregular	<1	White	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
T2	Irregular	<1	White	Opaque	Raised	Moist/Oily	Slimy	Rod	S-P-C	Pink	-	+	-
T3	Irregular	<1	White	Opaque	Convex	Moist/Oily	Slimy	Rod	S-P-C	Pink	-	+	-
O1	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
O2	Circular	<1	Cream	Opaque	Raised	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
O3	Irregular	<1	Cream	Opaque	Raised	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
O4	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
B1	Circular	<1	Cream	Opaque	Convex	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
B2	Circular	<1	Cream	Opaque	Convex	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
B3	Circular	<1	Cream	Opaque	Raised	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
S1	Circular	<1	White	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
S2	Circular	<1	Cream	Opaque	Raised	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
S3	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
S4	Circular	<1	White	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
W1	Circular	<1	Cream	Opaque	Raised	Smooth	Slimy	Rod	S-P-C	Pink	-	+	-
W2	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-
W3	Circular	<1	Cream	Opaque	Raised	Moist	Slimy	Rod	S-P-C	Pink	-	+	-

Key: P- Pineapple, T-Tomato, O-Orange, B-Banana, S-Sugarcane juice, W-Watermelon, + = positive, = negative S-P-C =single, pair, chain, <1 = less than, CT= Consistency, GS= Gram's stain reaction

Table 5 shows the important biochemical tests for acetic acid bacteria were carried out. All the isolates were found to be negative for oxidase, all isolates were found to be positive for catalase and positive for citrate. Their growth in the CARR medium showed the isolates T1, O3, and O4 had no over-oxidation, while the others showed over-oxidation. The isolates T1, O3, and O4 are probably *Gluconobacter* species, while the rest are probably *Acetobacter* sp.

Table 5: Biochemical Test for Isolated Acetic Acid Bacteria

Isolate	Oxidase	Catalase	Citrate	Reaction on Carr Medium	Probable Bacteria
P1	-	+	+	Overoxidation	*Acetobacter* sp
P2	-	+	+	Overoxidation	*Acetobacter* sp
P3	-	+	+	Overoxidation	*Acetobacter* sp
P4	-	+	+	Overoxidation	*Acetobacter* sp
T1	-	+	+	No overoxidation	*Gluconobacter sp*
T2	-	+	+	Overoxidation	*Acetobacter* sp
T3	-	+	+	Overoxidation	*Acetobacter* sp
O1	-	+	+	Overoxidation	*Acetobacter* sp
O2	-	+	+	Overoxidation	*Acetobacter* sp
O3	-	+	+	No overoxidation	*Gluconobacter* sp
O4	-	+	+	No overoxidation	*Gluconobacter* sp
B1	-	+	+	Overoxidation	*Acetobacter* sp
B2	-	+	+	Overoxidation	*Acetobacter* sp
B3	-	+	+	Overoxidation	*Acetobacter* sp
S1	-	+	+	Overoxidation	*Acetobacter* sp
S2	-	+	+	Overoxidation	*Acetobacter* sp
S3	-	+	+	Overoxidation	*Acetobacter* sp
S4	-	+	+	Overoxidation	*Acetobacter* sp
W1	-	+	+	Overoxidation	*Acetobacter* sp
W2	-	+	+	Overoxidation	*Acetobacter* sp
W3	-	+	+	Overoxidation	*Acetobacter* sp

Key: P- Pineapple, T-Tomato, O-Orange, B-Banana, S-Sugarcane juice, W-Watermelon, + = positive, -+negative

Table 6 shows the range of values for orange juice using *S. cerevisiae* in AAB1 set-up on day 1. The specific gravity is 1.065 ± 0.001, alcohol content is 0%, Brix is 15.8 ± 0.116, and pH is 3.9 ± 0.057. As the fermentation progressed to day 7, specific gravity decreased to 1.014 ± 0.001, alcohol increased to 3.9, brix decreased to 3.8 ± 0.288 and pH decreased to 3.0 ± 0.058. (Table 7). For sugarcane juice in the AAB2 setup, specific gravity is reduced from 1.9± 0.00 on day 1 to 1.006± 0.00 on day 7. Alcohol decreased from 0 on day 1 to 10.1 on day 7. Brix decreased from 18.9± 0.00 on day 1 to 1.6± 0.00 on day 7. pH decreased from 5± 0.00 on day 1 to 2± 0.00 on day 7. For pineapple in the AAB3 setup, specific gravity decreased from 1.074± 0.000 on day 1 to 1.020± 0.000 on day 7. Alcohol content increased from 0 on day 1 to 8.6 on day 7, and Brix reduced from 18.1± 0.000 on day 1 to 3.3 ± 0.00 on day 7. pH reduced from 3.9 ± 0.000 on day 1 to 2.9 ± 0.000 on day 7. For tomato in AAB4 set-up, specific gravity is reduced from 1.061± 0.000 on day 1 to 1.004± 0.000 on day 7. Alcohol increased from 0 on day 1 to 7.5 on day 7. Brix decreased from 15.0± 0.000 on day 1 to 1.1± 0.000 on day 7. pH decreased from 4.1± 0.000 on day 1 to 3.2± 0.000 on day 7. 1 to 1.080±0.000 in week 4.

Table 6: Physicochemical Parameters and Alcohol Yield from Fermentation of Juices by *Saccharomyces cerevisiae*

Juice (Set-up)	Days	Specific Gravity	º Brix	pH	Alcohol (%)
Orange	1	1.065± 0.001^{f}	15.8 ± 0.116^{f}	3.9 ± 0.057^{c}	0
(AAB1)	2	1.061± 0.001^{e}	14.9 ± 0.23^{e}	3.9 ± 0.000^{c}	0.6
	3	1.061± 0.000^{e}	14.8 ± 0.00^{e}	3.8 ± 0.058^{c}	0.7
	4	1.051 ± 0.002^{d}	12.7 ± 0.360^{d}	3.6 ± 0.058^{b}	2.9
	5	1.037 ± 0.001^{c}	9.7 ± 0.289^{c}	3.5 ± 0.000^{b}	4.0
	6	1.020 ± 0.001^{b}	5.1 ± 0.577^{b}	3.1 ± 0.116^{a}	6.2
	7	1.014 ± 0.001^{a}	3.8 ± 0.288^{a}	3.0 ± 0.058^{a}	3.9
Sugarcane	1	1.9± 0.000^{g}	18.9± 0.000^{g}	5± 0.000^{b}	0
(AAB2)	2	1.06± 0.000^{f}	14.9± 0.000^{f}	4.9± 0.000^{b}	4.6
	3	1.047± 0.000^{e}	11.67± 0.000^{e}	4.8± 0.000^{b}	4.6
	4	1.04± 0.000^{d}	10.1± 0.000^{d}	4.5± 0.000^{b}	5.5
	5	1.024± 0.000^{c}	6.1± 0.000^{c}	4.4± 0.000^{b}	7.7
	6	1.013± 0.000^{b}	3.3± 0.000^{b}	4.1± 0.000^{b}	9.4
	7	1.006± 0.000^{a}	1.6± 0.000^{a}	2± 0.000^{a}	10.1
Pineapple					
(AAB3)	1	1.074± 0.000^{f}	18.1± 0.000^{g}	3.9± 0.000^{d}	0
	2	1.065± 0.000^{e}	16± 0.000^{f}	3.9± 0.000^{d}	1.4
	3	1.063± 0.000^{d}	13.4± 0.000^{e}	3.6± 0.000^{c}	3
	4	1.040± 0.000^{c}	10.1± 0.000^{d}	3.4± 0.000^{b}	4.9
	5	1.028± 0.000^{b}	7.4± 0.000^{c}	3.1± 0.000^{a}	6.4
	6	1.016± 0.000^{a}	4.8± 0.000^{b}	3± 0.000^{a}	7.8
	7	1.020± 0.000^{a}	3.3± 0.000^{a}	2.9± 0.000^{a}	8.6
Tomato					
(AAB4)	1	1.061± 0.000^{f}	15.0± 0.000^{f}	4.1± 0.000^{e}	0
	2	1.057± 0.000^{f}	14.0± 0.000^{f}	4.0± 0.000^{e}	0.3
	3	1.053± 0.000^{e}	13.1± 0.000^{e}	3.9± 0.000^{d}	1.2
	4	1.044± 0.000^{d}	10.9± 0.000^{d}	3.7± 0.000^{c}	2.3
	5	1.032± 0.000^{c}	8.0± 0.000^{c}	3.6± 0.000^{c}	3.7
	6	1.012± 0.000^{b}	3.1± 0.000^{b}	3.4± 0.000^{b}	6.5
	7	1.004± 0.000^{a}	1.1± 0.000^{a}	3.2± 0.000^{a}	7.5

Values are Mean of triplicate ± Standard Deviation, Alcohol in percentage
Values with different superscript within the same column have significant difference ($p < 0.05$)

Table 7 presents the following data. For orange, pH decreased from 3.0 ±0.000 in week 1 to 2.2 ± 0.116 in week 4. The volume of NaOH used increased from 5.2 in week 1 to 11.2 in week 4. Titratable acidity increased from 3.1 ±0.058 in week 1 to 6.7 ±0.058 in week 4. Specific gravity decreased from 1.016 ±0.001 in week 1 to 1.090 ±0.001 in week 4. Brix decreased from 4.2±0.116 in week 1 to 0.0±0.000 in week 4. For sugarcane juice, pH decreased from 4.0±0.0578 in week 1 to 2.9±0.058 in week 4. The volume of NaOH used increased from 3.7±0.000 in week 1 to 11.0±0.000 in week 4. Titratable acidity increased from 2.2 ±0.000 in week 1 to 6.6±0.000 in week 4. Specific gravity decreased from 1.005±0.001 in week 1 to 1.085±0.000 in week 4. Brix decreased from 1.2

±0.173 in week 1 to 0.0±0.000 in week 4. For spoilt pineapple juice, pH decreased from 2.9±0.058 in week 1 to 2.3 ±0.208 in week 4. The volume of NaOH used increased from 3.7±0.000 in week 1 to 12.9±0.17 in week 4. Titratable acidity increased from 2.2±0.000 in week 1 to 7.7±0.116 in week 4. Specific gravity decreased from 1.005±0.001 in week 1 to 1.104±0.059 in week 4. Brix decreased from 1.2±0.173 in week 1 to 0.0±0.000 in week 4. Tomato juice, pH decreased from 3.2±0.000 in week 1 to 2.6±0.058 in week 4. The volume of NaOH used increased from 4.2±0.058 in week 1 to 11.2±0.00 in week 4. Titratable acidity increased from 2.5±0.000 in week 1 to 6.7±0.000 in week 4. Specific gravity decreased from 1.004±0.000 to 1.080±0.000 in tomato. Brix decreased from 1.0 ±0.000 in week 1 to 0.0±0.000 in week 4.

Table 7: Physicochemical Properties of Juices Fermented by *Acetobacter* species

Juice (Set-up)	Weeks	pH	Vol of NaOH	Titratable Acidity(%)	Specific Gravity	^{o} Brix
Orange(AAB1)	1	3.0 ±0.000^{d}	5.2 ± 0.058^{d}	3.1 ±0.058^{d}	1.016 ±0.001^{d}	4.2±0.116^{c}
	2	2.8 ±0.058^{c}	7.7 ±0.000^{c}	4.6 ±0.000^{c}	1.007 ±0.001^{c}	1.7±0.173^{b}
	3	2.5 ±0.000^{b}	10.1±0.116^{b}	6.1 ±0.058^{b}	1.061 ±0.001^{b}	0.0±0.000^{a}
	4	2.2 ± 0.116^{a}	11.2±0.058^{a}	6.7 ±0.058^{a}	1.090 ±0.001^{a}	0.0±0.000^{a}
Sugarcane(AAB2)	1	4.0±0.0578^{d}	3.7±0.000	2.2 ±0.000	1.005±0.001^{d}	1.2 ±0.173^{b}
	2	3.6±0.116^{c}	9.0±0.000	5.4±0.000	1.044±0.002^{c}	0.0 ±0.000^{a}
	3	3.0±0.000^{b}	10.0±0.000	6.0±0.000	1.063±0.001^{b}	0.0 ±0.000^{a}
	4	2.9±0.058^{a}	11.0±0.000	6.6±0.000	1.085±0.000^{a}	0.0 ±0.000^{a}
Pineapple (AAB3)	1	2.9±0.058^{b}	3.7±0.000^{d}	2.2±0.000^{d}	1.005±0.001^{b}	1.2±0.173^{b}
	2	2.6 ±0.058^{a}	8.5±0.000^{c}	5.1±0.000^{c}	1.039±0.001^{a}	0.0±0.000^{a}
	3	2.4 ±0.116^{a}	11.5±0.00^{b}	6.9±0.000^{b}	1.000±0.000^{a}	0.0±0.000^{a}
	4	2.3 ±0.208^{a}	12.9±0.17^{a}	7.7±0.116^{a}	1.104±0.059^{a}	0.0±0.000^{a}
Tomato (AAB4)						
	1	3.2±0.000^{d}	4.2±0.058^{d}	2.5±0.000^{d}	1.004±0.000^{c}	1.0±0.000^{b}
	2	3.0±0.058^{c}	6.3±0.153^{c}	3.8±0.100^{c}	1.011±0.001^{b}	0±0.000^{a}
	3	2.8±0.058^{b}	9.4±0.116^{b}	5.6±0.058^{b}	1.048±0.000^{a}	0±0.000^{a}
	4	2.6±0.058^{a}	11.2±0.00^{a}	6.7±0.000^{a}	1.080±0.000^{a}	0±0.000^{a}

Values are Mean of triplicate ± Standard Deviation, Alcohol in percentage
Values with different superscript within the same column have significant difference ($p < 0.05$)

Shown in Figure 2 is the data for sensory evaluation of crude vinegar, produced from orange, sugarcane, tomato, and pineapple. The range of data for pungency, pineapple is highest with 6.8 and orange with 5.7. For Colour, pineapple was highest with 7.4, tomato and orange each had 6.8. For aroma, Pineapple is highest at 7.9, sugarcane at 7.2, tomato at 6.5, and orange at 6.4. For taste pineapple has the highest value with 7.4, followed by sugarcane with 7.0, tomato with 6.6, and orange with 5.8. For general acceptability, pineapple is the highest with 7.9, followed by sugarcane with 7.6, orange with 7.2, then tomato with 6.9.

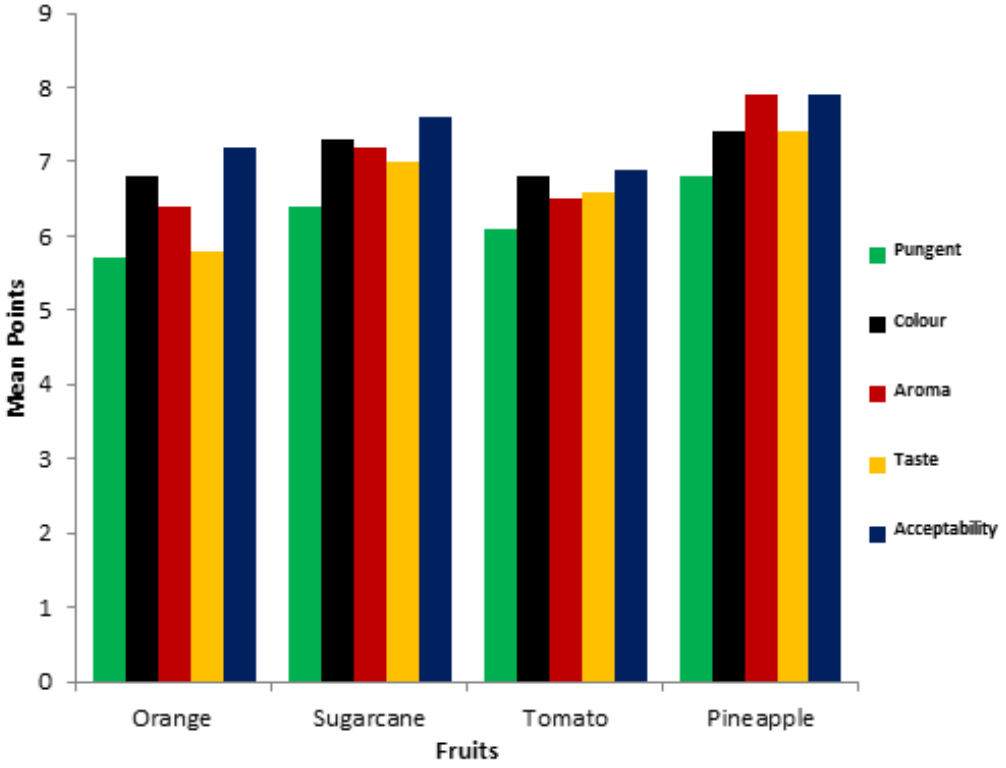

Figure 2: Sensory Evaluation of Produced Vinegar

In Table 8, the antibacterial effect of crude vinegar on cabbage in colony-forming unit per milliliter is presented. In 0 min, the bacterial count was 2.07×10^5 for the untreated cabbage 0% (v/v), 1.07×10^5 for 5% (v/v), 7.0×10^4 for 10%(v/v) and 5.4×10^4 for 15%(v/v). In 5 min, the bacterial count was 1.82×10^5 for the untreated cabbage 0% (v/v), 7.9×10^4 for 5% (v/v), 4.5×10^4 for 10%(v/v) and 3.8×10^4 for 15%(v/v). In 10 min, the bacterial count was 1.59×10^5 for the untreated cabbage 0% (v/v), 6.4×10^4 for 5% (v/v), 3.4×10^4 for 10%(v/v) and 2.7×10^4 for 15%(v/v).

Table 8: Antibacterial Effect of Crude Vinegar on Cabbage (CFU/g)

Time (Min)	Untreated	Treated with Vinegar (v/v)		
	Water	5%	10%	15%
0	$2.07\times10^5 \pm 4.56^a$	$1.07\times10^5 \pm 6.42^b$	$7.0\times10^4 \pm 2.65^c$	$5.4\times 10^4 \pm 5.29^d$
5	$1.82\times10^5 \pm 1.00^a$	$7.9\times10^4 \pm 1.00^b$	$4.5\times10^4 \pm 1.53^c$	$3.8\times 10^4 \pm 1.53^d$
10	$1.59\times10^5 \pm 2.08^a$	$6.4\times10^4 \pm 3.21^b$	$3.4\times10^4 \pm 3.21^c$	$2.7\times 10^4 \pm 2.10^d$

Values are Mean of triplicate ± Standard Deviation
Values with different Superscript across the rows are statistically different

Presented in Table 9, is the antifungal activity of crude vinegar on cabbage in colony-forming unit per milliliter. At 0 min, at 5 min, and 10 min. In 0 min, the fungal count was 4.9×10^4 for the untreated cabbage 0% (v/v), 3.1×10^4 for 5% (v/v), 2.9×10^4 for 10%(v/v) and 1.47×10^4 for 15%(v/v). In 5 min, the fungal count was 4.1×10^4 for the untreated cabbage, 0% (v/v), 1.5×10^4 for 5% (v/v), 1.0×10^4 for 10%(v/v) and 8.7×10^3 for 15%(v/v). In 10 min, the fungal count was 3.1×10^4 for the untreated cabbage 0% (v/v), 1.0×10^4 for 5% (v/v), 8.0×10^3 for 10%(v/v), and 6.7×10^3 for 15%(v/v).

Table 9: Antifungal Effect of Crude Vinegar on Cabbage (CFU/g)

Time (Minute)	Untreated	Treated with Vinegar (v/v)		
	Water	5%	10%	15%
0	$4.9\times 10^4 \pm 4.40^d$	$3.1 \times 10^4 \pm 3.00^c$	$2.9\times 10^4 \pm 3.51^b$	$1.47\times 10^4 \pm 2.10^a$
5	$4.1\times 10^4 \pm 3.21^c$	$1.5\times 10^4 \pm 1.00^b$	$1.0\times 10^4 \pm 1.0^a$	$8.7\times 10^3 \pm 3.05^a$
10	$3.1\times 10^4 \pm 1.53^b$	$1.0\times 10^4 \pm 2.10^a$	$8.0\times 10^3 \pm 2.65^a$	$6.7\times 10^3 \pm 2.89^a$

Values are Mean of triplicate ±Standard Deviation

DISCUSSION

The moisture content of all juices was relatively high, up to 82.2% in sugarcane. Tomato juice had the lowest moisture content, likely due to the breakdown of cell walls, which release water into the juice, when it was sorted as a surplus. Igile *et al.* (2016) reported a lower moisture content of healthy tomato juice at 48.0%. The ash content of all the juices was relatively low. Ash is made up of soluble inorganic minerals such as calcium, phosphorus, and potassium. Sugarcane juice had the lowest ash content of 0.4%. Obassa *et al.* (2022) reported the ash content of pineapple to be 0.15%. The lipid content of the juices was relatively low, ranging from 7.3% to 13.3% for sugarcane and tomato due to their location in the cell membranes, which are broken down during spoilage. The protein content of the juices was relatively low, ranging from 1.1% to 2.8% for sugarcane and orange. Oladipo *et al.* (2022) reported a higher protein content of 11.38% and 12.25% for orange and pineapple, respectively. This depends on their location in the cell membrane. Tekalign and Fistum (2017) reported that pineapple had a higher fiber content of 12% and orange had 2.4%. Sugarcane juice had the highest carbohydrate content because carbohydrate content is affected by the type of juice and the ripeness of the juice. (A.O.A.C., 2010; Konadu *et al.*, 2021).

The results of reducing sugar content showed that sugarcane juice had the highest optical density, followed by pineapple juice, orange juice, and tomato juice. Orji *et al.* (2015) reported a close value for pineapple at 1.53 mg/mL. This suggests that sugarcane juice has the highest concentration of reducing sugar, followed by pineapple juice, orange juice, and tomato juice. According to Zabed *et al.* (2014), the content of fermentable sugar in sugarcane juice is about 12–17%, of which 90% is sucrose and the remaining 10% is glucose and fructose.

Saccharomyces cerevisiae was identified. All the yeast strains isolated from grape juice in this work were similar to those isolated by Chatterjee *et al.* (2011). Yeast cells grow distinctively on solid media because their individual species reproduce through budding and form distinct colonies that are typically small in size and creamy in appearance (Prescott *et al.*, 2008). Grape juice contains sugar, and yeasts are mostly found in areas where fermentation can occur. Yeast is a critical component in the fermentation process that converts sugar into alcohol, an ingredient present in wine and distilled beverages (Tumane *et al.*, 2018). The microscopic observation of the yeast cells shows that they are single cells, oval or spherical in shape, and budding. Budding is their method of reproduction. This is similar to the finding of Karki *et al.* (2017).

Saccharomyces cerevisiae was able to ferment glucose, sucrose, fructose, maltose, mannitol, and peptone. This is due to the presence of enzymes called zymases. Zymases are a group of enzymes that catalyze the breakdown of carbohydrates into alcohol and carbon dioxide (Dash *et al.*, 2015; Sudeepa and Sanja, 2020). The gas observed in the Durham tubes was carbon dioxide, which is a byproduct of fermentation, and the change of the indicator color of the basal media from red to yellow was due to the reduction of pH. Lactose, mannitol, and xylose were not used up by *S. cerevisiae*, as reported by Kechkar *et al.* (2019). All the isolates of acetic acid bacteria were able to utilize glucose, sucrose, and fructose, as similarly reported by Ouattara *et al.* (2019) and Srivastava and Rani (2019). They all grew in xylose except T2 and S4. All utilized mannitol except P3, P4, T2, O1, O2, and B3. None of the isolates were able to

utilize lactose or maltose. Xylose requires the enzyme xylose isomerase to be converted to a form that is usable by acetic acid bacteria. Lactose is a disaccharide; it requires the enzyme lactase (beta-galactosidase) to be broken down into its two components, glucose and galactose. Maltose is also a disaccharide, which requires the enzyme maltase to be broken down into its two glucose components (Prescott *et al.*, 2008). The ability of the yeast to tolerate up to 20% alcohol with low growth is due to the presence of an enzyme called alcohol dehydrogenase. Alcohol dehydrogenase is an enzyme that catalyzes the oxidation of alcohol into acetaldehyde. Acetaldehyde is then further metabolized to acetic acid. The acetic acid produced by the yeast is responsible for the sour taste of alcoholic beverages (Sudeepa and Sanja, 2020).

In acetic acid bacteria, at 4%, there was intensive growth of all isolates. There was moderate growth at 6% concentration and low growth at 8% concentration of alcohol due to the inability of some isolates to produce the alcohol dehydrogenase enzyme. The lowest growth was observed at 10%, and only four isolates survived, namely: P1, T3, O2, and S1, which were named AAB1, AAB2, AAB3, and AAB4, respectively.

Membrane-bound alcohol dehydrogenase enzymes are the enzymes that catalyze the oxidation of alcohols to aldehydes. In acetic acid bacteria, they are located in the cell membrane, which allows them to oxidize alcohols that are present in the external environment. Furthermore, the tricarboxylic acid cycle, pyrroquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH), and acetaldehyde dehydrogenase (PQQ-ALDH) activities had the potential to adapt to high ethanol challenges, with PQQ-ADH activity contributing more to the ethanol tolerance. The results obtained reveal that the characteristic membrane structure, energy metabolism, and their improved adaptive regulation contributed to the high ethanol tolerance of AAB (Hu *et al.*, 2022). The isolates that survived in 10% alcohol are rich in alcohol dehydrogenase enzymes in their membranes (Cleenwerck, 2013). Additionally, the results suggest that 10% alcohol can be used to inhibit the growth of acetic acid bacteria, which could be useful for food safety purposes (Zheng *et al.*, 2015).

The cells appear pink in color, indicating that they are gram-negative, and their cell wall is made up of a thick peptidoglycan layer, which was able to retain the counterstain (Ezemba, 2022). The cells are motile, indicating the presence of flagella or other motility structures. However, they do not form spores. All of these characteristics are consistent with the characteristics of acetic acid bacteria. They agree with the findings of Fu *et al.* (2013), Kowser *et al.* (2015), Tharinee *et al.* (2015), and Ballankimath *et al.* (2017).

Onuorah *et al.* (2016) recorded all negatives for the oxidase enzyme; the same was obtained in this study. Oxidase enzymes play an important role in the operation of the electron transport system during aerobic respiration (Tumane *et al.*, 2018). This is consistent with the fact that acetic acid bacteria are gram-negative bacteria. Gram-negative bacteria typically do not produce oxidase (Prescott *et al.*, 2008). All isolates were positive for catalase, as was also reported by Arifuzzaman *et al.* (2014). These acetic acid bacteria contain flavoproteins that reduce oxygen, resulting in the production of hydrogen peroxide, or superoxide. All isolates were positive for citrate, indicating the ability to utilize citrate. This is a characteristic of many gram-negative bacteria (Sagar, 2019). Citrate is a type of organic acid that can be used as a carbon source by bacteria (Ezemba, 2022). The colonies on GYC agar appeared to have a clear zone formation around them due to the disappearance of $CaCO_3$. The disappearance of $CaCO_3$ and the formation of a clear zone around the growing colony were due to the production of acetic acid, which reacts with $CaCO_3$ and produces calcium acetate, which is water-soluble. A similar selection method was used by Arifuzzaman *et al.* (2014). Carr medium is composed of yeast extract, agar, alcohol, and an indicator (bromophenol blue). This indicator changes color from blue to yellow at an acidic pH (Romero-Cortes *et al.*, 2012). Acetic acid bacteria produce acetic acid, which lowers the pH of the medium, causing the indicator to change color. The color changed from blue to yellow at oxidation, but over-oxidation made it change back to blue, as was observed in suspected *Acetobacter* species after 30 min. Carr

medium is used to distinguish between *Acetobacter* and *Gluconobacter* species. The difference between oxidation and overoxidation is; oxidation is the process of converting alcohol to acetic acid, while overoxidation is the conversion or breakdown of acetic acid to carbon dioxide and water (Yanti *et al.*, 2107). The result of this screening showed that the isolates T1, O3, and O4 did not overoxidize, while the other isolates did. This suggests that the isolates T1, O3, and O4 are probably *Gluconobacter* species, while the other isolates are probably *Acetobacter* species. The overoxidation of ethanol to acetic acid is a characteristic of *Acetobacter* species, as described by Sharfi *et al.* (2010).

Fermentation of juices by *Saccharomyces cerevisiae* showed a similar trend with Ho *et al.* (2017a). The decrease in specific gravity over time indicates that the fermentation process is converting the sugars in the juice into alcohol (Braide *et al.*, 2011). The decrease in pH over time indicates that the fermentation process is producing acids. A similar trend was observed by Onuorah *et al.* (2016). The alcohol content of the fermented juices was lowest in orange juice and highest in sugarcane juice. This is because sugarcane juice has a higher sugar content than orange juice, so it produces more alcohol during fermentation. The increase in alcohol content of orange was similar to that of Cejundo-Bastane *et al.* (2016), who observed 5% in 4 d. Brix grades are closely related to alcoholic fermentation; they represent the concentration of sugars (Luzon-Quintana *et al.*, 2021).

The second stage of fermentation by *Acetobacter* species shows a decrease in pH, an increase in the volume of NaOH used up in titration, an increase in titratable acidity, a decrease in specific gravity, and a decrease in degree brix. Pineapple in AAB3 had the highest titratable acidity of 7.7%, followed by orange, tomato, and sugarcane. Patel and Pandya (2015) reported that an increase in pH decreases the production of acetic acid due to inhibition by metabolites, and their best results were obtained at pH 4.5, where maximum production was noticed, and then a further increase in pH decreases the production. The volume of NaOH is the amount of NaOH used to titrate the acetic acid contained in crude vinegar. The research of Chen *et al.* (2015) reports a similar trend to this study's sugarcane vinegar. Pineapple juice in the setup using AAB3 produced the highest acetic acid in this entire study. Its final titratable acidity was 7.7%. Raji *et al.* (2014) and Tumane *et al.* (2018) produced acetic acid from pineapple peels at 4.6% and 4.7%, respectively. This could be due to the effects of pretreatment of cellulose (Roda *et al.*, 2014). According to the report by Selvanathan *et al.* (2020), acetic acid production slightly decreased over fermentation time, the reason being that acetic acid evaporates easily. Ezenekwe *et al.* (2021) also recorded an increase in the titratable acidity of the vinegar produced with an increase in fermentation time. It is also worth noting that other compounds, such as esters and aldehydes, are produced. These compounds contribute to the flavor of vinegar (Bhat *et al.*, 2014). The fermentation process can be controlled to produce vinegar with different acetic acid contents. For example, the fermentation process can be stopped early to produce vinegar with a lower acetic acid content. The fermentation process can also be continued for a longer period to produce vinegar with a higher acetic acid content. This result agrees with the work of Ezenekwe *et al.* (2021). Onuorah *et al.* (2016) also stated that the standard acetic acid content of vinegar by the Standards Organization of Nigeria is 4%–8%.

In Figure 2, vinegar produced from pineapple was the most preferred by the panelists. It had the highest scores for all qualities. Ezenekwe *et al.* (2021) reported high sensory qualities in pineapple. The high scores for pineapple vinegar could be attributed to its unique flavor and aroma. It is also rich in nutrients, which makes it more preferred by consumers (Huang *et al.*, 2017; Tumane *et al.*, 2018; Obassa *et al.*, 2022). Sugarcane vinegar was the second most preferred, corroborating Chen *et al.* (2015). Beltran-Milan (2016) reported that it has a sweet and tart flavor. Results from Perumpulli *et al.* (2022) showed that all parameters in tomato vinegar were lower than this study and are a good choice for those who prefer a savory flavor. Orange vinegar is a good choice for those who prefer a milder flavor.

The research of Kabir *et al.* (2022) showed that vinegar has bacteriostatic and bactericidal effects on microorganisms. In this study, the bacterial count continued to decrease with an increase in

the concentration of vinegar and an increase in time. This was because the vinegar had more time to reduce the bacterial load. This result agrees with the studies of Atter *et al.* (2014), Da Silva *et al.* (2016), Kyari *et al.* (2022), and Traore *et al.* (2022). Hiroaki (2015) explained that the antimicrobial activity of acetic acid is partly due to the lowering of pH in the environment. Although the acetic acid produced becomes stressful for even the acetic acid bacteria themselves, they can be grown under highly concentrated acetic acid conditions.

This research shows that fungal inhibition increases with an increase in acetic acid concentration, and the efficacy of treatment depends on exposure time and concentration. The results of the antifungal properties of vinegar correspond with the findings of Bayram *et al.* (2020) and Kabir *et al.* (2022).

CONCLUSION

In conclusion, the results from this study show the successful production of vinegar from the juices of farm surpluses, namely pineapple, sugarcane, tomato, and orange, using *Acetobacter* species isolated from juices. The physicochemical and proximate composition analyses provided insights into the properties and nutritional composition of the juices. The isolation and identification of *Acetobacter* species and *Saccharomyces cerevisiae* confirmed their presence and suitability for fermentation. Double-stage fermentation resulted in the conversion of sugars into ethanol and subsequently into acetic acid, leading to the production of vinegar. Sensory evaluation revealed favorable taste, aroma, and overall acceptability, indicating distinct qualities. Vinegar proved to be an effective antimicrobial agent, useful in food preservation. These findings contribute to the development of sustainable waste management practices and biotechnology in the food industry. Future research should focus on process optimization and gene engineering.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial or personal interests that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

P.S.K. conceptualized and designed the experiments, research, and manuscript preparation. W.J.R., conducted data analysis, interpretation of results, and manuscript preparation. O.A.A. Contributed materials. All authors carried out laboratory experiments. All the authors read and approved the final manuscript.

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