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Production and Comparative Physicochemical Analysis of Vinegar from Locally Grown Fruits in Nigeria and Industrial Produced Vinegar

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Abstract Vinegar is the product made from the conversion of ethyl alcohol to acetic acid by a genus of bacteria, *Acetobacte*r. The major aim of the work is to produce a vinegar with locally made fruits and compare it with the industrial produced vinegar to ascertain if it can be used to substitute the industrial produced vinegar and to also determine if there are presence of acetic acid bacteria present in the vinegar which can be used as inoculants for future fermentation the sensory attributes were also analysed to determine the consumer acceptability of the vinegar produced. This work involves production of vinegar from locally produced fruits in Nigeria by homemade scale process and then analyzing the vinegar to determine the physicochemical components of the vinegar as well as identifying the acetic acid bacteria present in the vinegar and its sensory attributes. The vinegar produced was analysed for its pH, relative density and titrable acidity and from the result, there was no significant difference in the pH and relative density of the vinegars. The titrable acidity of the industrial produced vinegar is higher than the locally produced vinegars analysed. The microbial isolation of the vinegar for safety assessment gave no growths in the media used for the analysis which means that there are no pathogenic organisms like mould or coliform which can cause diseases to the consumers. The major organism isolated was the *Acetobacter spp*. although the culture independent methods should be carried out to determine other non culturable organisms present in the vinegar. The sensory evaluation shows that the locally produced was well accepted from the scores on the result presented. Generally, the result of this work shows that indeed vinegar of good quality can be produced locally using locally grown fruits but optimization of the process should be carried out to improve yield.

Keywords: *vinegar, consumer acceptability, titrable acidity, physicochemical analysis, safety, sensory evaluation*

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1. Introduction

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" [1]. Vinegar is the product made from the conversion of ethyl alcohol to acetic acid by a genus of bacteria, *Acetobacte*r [2]. Vinegar is a condiment, made from various sugary and starchy materials by alcoholic and subsequently, acetic acid fermentation [3,4].

Vinegar is of two type i.e. synthetic (non-brewed) vinegar and brewed (natural) vinegar. Brewed vinegar is prepared from any sugary material like sugarcane juice, jaggery, palm juices, grapes, apple and molasses. Synthetic vinegar is made from acetic acid and is not fermented/ brewed. Brewed vinegar is produced from the substrates like honey, sugarcane, grape, apple, plum, coconut and jamun etc. Wine (white, red, and sherry wine), cider, fruit wines, malted barley, or pure alcohol have also been used as substrates for vinegar production. In recent years, researchers have also produced natural vinegar from sources such as cashew, Indian jujube (*Zizyphus mauritiana)* and pineapple [5]. Vinegar is also made from the apple juice or concentrated apple juice [6,7].

Vinegar contains 4 % acetic acid that is produced from sugary materials through alcoholic fermentation [8]. Acetic acid is the predominant flavoring and antimicrobial component in vinegar. Vinegar industry produces several types of vinegar by various qualified native or engineered acetic acid bacteria [8].

Vinegar may be produced from a variety of raw materials, the main requirement being satisfactory economic source of ethanol. 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

[9] or any other sugary food [10]. The common types of vinegar in a region often reflect the local alcoholic beverage (e.g., rice vinegar is popular in Japan, wine vinegar in France, and malt vinegar in the UK) [11,12]. There are many types of vinegars. The classification is usually based on the raw materials used for its production and includes malt vinegar, wine vinegar, apple cider vinegar, balsamic vinegar, fruit vinegar etc. [13]. Although vinegar has always been considered among the lowest quality products of fermented foods, it has also been used as a food condiment, as a preservative agent and, in some countries as a healthy drink [14].

Productions of vinegar are mostly carried at home scale/ cottage industry using natural fermentation. With respect to cider vinegar, the vinegar made from apple, the acetification is also carried out naturally but is a slow process. The acetic acid bacteria in pure culture have also been employed but the performance is always lower. Therefore, most of the fermentations employ consortia or natural culture or mixed acetic acid bacteria. So, there is a need to standardize the culture of bacteria. Industrial vinegar manufacturing processes fall into three main categories: slow processes, quick processes, and submerged processes [12]. General production method can range from traditional method for example wood casks (Orleans process) and surface culture (generator process) to submerged fermentation method [15].

Microbial species involved in fermentations may range from yeast and lactic acid bacteria (LAB) to molds and acetic acid bacteria (AAB). The microorganisms involved in the elaboration of vinegars are mainly yeasts and AAB. The former being responsible for the alcoholic fermentation and the latter needed for the acetification [16,17,18]. Yeasts are the most important microorganisms during alcoholic fermentation because they influence fermentation speed, wine flavor and other wine qualities [19]. Joshi *et al.*, [20] studied that for cider production, the strains commonly used belonged to the species *Saccharomyces cerevisiae* or *Saccharomyces bayanus* and the choice of yeast strain as starting culture could have a high impact on the flavor profile of fermented beverages. Although a variety of bacteria can produce acetic acid, mostly members of *Acetobacter* (Gluconoacetobacter) are used commercially, typically the aerobic bacterium *A. aceti* at 27°C - 37°C [11,12,21,22]. Other species frequently isolated from vinegar fermentations include *A Acetobacter pasterianus*, *Acetobacter polyoxogenes*, *Gluconacetobacter xylinus*, *Gluconacetobacter hansenii*, *Gluconacetobacter oboediens*, and *Gluconacetobacter intermedius* [23,24]. The most important properties of a production strain in the vinegar industry are tolerance to high concentrations of acetic acid and total concentration, low nutrient requirements, inability to over oxidize the formed acetic acid, high production rate, and resistance to phage infections. The trend in consuming vinegar in Nigeria is on the increase, and in as much as so many benefits have been observed in the use of vinegar, little attention has been given to the locally produced vinegar from locally grown fruits. It is therefore for this reason that up till this moment, we still deal on industrially produced vinegar whose nutritional facts are yet to be verified and also very expensive, It is necessary therefore to access the locally grown fruits in vinegar production as well as solve issues relating to expense and consequently improve the economy of the country since these fruits whether spoilt or fresh is a great substrate for vinegar production. The aim of this work is to determine the vinegar production ability of some locally grown fruits and comparison of the locally produced and industrially produced vinegar and isolation of acetic acid bacteria which can be used as inoculants or starters for future fermentation.

2. Material and Methods

2.1. Production of the Vinegar Samples

Fruits like apple, pawpaw, jack fruit, pineapple, oranges, lemon, lime and grape was bought from Ukwuorji, Permanent Site market Awka. The Fruits were gathered and washed. Twenty grams (20 g) of different fruits were weighed, diced, soaked in distilled water, and allowed to ferment naturally at room temperature in 500 mL of conical flask. The distilled water was poured to about three-quarters capacity of the flask, corked with cotton wool for 7 days and stirred daily. During this period, the mixture ferments into alcohol. The mixture was decanted and poured into a bottle. The mixture was allowed to sit open at room temperature for several weeks, blended, inoculated with mother (a slimy membrane composed of cells of microorganisms found on the surface of alcoholic liquids undergoing acetous fermentation and can be added to cider or wine to produce vinegar),from previously fermented vinegar and allowed to ferment. The mixture is then transferred into a larger glass container (1L) and covered with cheese cloth. The bottles were placed in the dark. The fermentation was allowed for 3 months with monitoring and continuous check of pH, titrable city and relative density was carried out on interval days. On the 90^{th} day, the products were filtered using a tea strainer to remove the produced slime before physicochemical analysis and sensory evaluation. The industrial produced vinegar was Bragg apple cider vinegar got from a supermarket in Awka, Anambra state, Nigeria.

2.2. Acetic Acid Bacteria Isolation from the Locally and Industrial Produced Vinegar

AAB will be isolated by plating samples on Glucose-Yeast extract-Calcium carbonate (GYC) broth at an adequate dilution, supplemented with natamicine (100 mg/L) at 37°C. GYC broth was used as enrichment medium. This is the medium used for the growth and maintenance of *Acetobacter spp* [25]. Spread plate technique was followed throughout this study. 0.1mls was of the sample was transferred on GYC media (10% glucose, 1% yeast extract, 2% $CaCO_3$, 1.5% Agar) and incubated at 30°c for 48 hours. Diluted samples of 0.1 ml were transferred with sterile pipette and spreaded on GYC agar plates with the help of sterile bent rod in a sterile condition. All the GYC plates were incubated in an incubator at 30°C for 48 hours. To confirm the acid production, formation of a halo around the colony will be examined. Then single morphology well-formed colonies were isolated and sub cultured by streak plate technique

on GYC agar plates to check its purity and incubated at 30°C for 48 hours. Gram's staining, sugar fermentation methyl red test, Voges-Proskauer, motility test, catalase test, indole and oxidase test was carried out on the isolates.

2.3. Microbial Evaluation of the Local and Industrial Produced Vinegar

2.3.1. Microbial Isolation for Safety Assessment

This analysis involves evaluation of the vinegar samples to determine the total viable count, the molds and coliform count using their respective media.

2.3.2. Enumeration of the Total Viable Count

ISO 4833-1: 2013 [26] method was used in this evaluation. The required amount of molten plate count agar was prepared and held at water bath maintained at 48°C±2°C. The work benches were cleaned with 70% alcohol and the gas flame was also lighted. 1 ml of the vinegar samples were introduced into 9mls of peptone water and homogenized. 1ml volume of the homogenyized sample was inoculated into the petridishes and molten plate count agar was gently poured in to the petri plates and rocked gently to mix. The plates were allowed to solidify and aerobically incubated at 30°C for 72hrs. After incubation, colonies formed on the agar plates were counted.

2.3.3. Enumeration of the Coliform Count

1SO4832-2006 [53] method was used in this evaluation. The required amount of the molten Congo red agar was prepared and held at water bath maintained at 48°C±2°C. The work benches were cleaned with 70% alcohol and the gas flame was also lighted. 1 ml of the vinegar samples were introduced into 9mls of peptone water and homogenized. 1ml volume of the homogenyized sample was inoculated into the petridishes and molten Congo red agar was gently poured in to the petri plates and rocked and incubated for 24hrs ±2 at 30°C. After incubation, colonies formed on the agar plates were counted.

2.3.4. Enumeration of the Mold Count

The spread plate method was used. 1% of the sample was diluted in peptone water broth. Potato dextrose agar supplemented with Chloramphenicol was used in the evaluation. The agar was prepared, poured in plates and allowed to solidify. 1ml of the test sample was transferred into the medium and spread over the surface of the agar plate with a sterile spreader until the liquid is completely absorbed into the medium and incubated aerobically in an upright position in the incubator at 25°C ±1°C for for 5days. After incubation, colonies formed on the agar plates were counted [27].

2.4. Physicochemical Quality Evaluation

Determination of pH: the pH was determined according to methods of Association of Analytical Chemists (2000). Ten millilitres (10 mL) of the vinegar will be weighed into a beaker, mixed thoroughly in 100 ml of distilled water and centrifuged for 20 mins at 200 rpm. The supernatant will be decanted and the pH determined using a standard pH meter.

Determination of specific gravity: Density bottle was washed, oven dried, cooled and weighed using a weighing balance. The sample was filled into the density bottle and weighed. Distilled water was then filled into bottle and weighed. The specific gravity (SG) was calculated as

$$SG = \frac{Weight\ of\ sample}{Weight\ of\ Water}$$ [28]

Determination of titrable acidity: The content of acid in the vinegar was analysed titrimetrically. The titration was made with 0.1 M NaOH and the acidity was calculated as acetic acid in vinegar [28].

$$\%TTA(wt/wt) = \frac{N \times V \times Eqwt}{W \times 1000} \times 100$$

Where;
N = normality of titrant, usually NaOH (mEq/ml)
V = volume of titrant (ml)
MWacetic acid is acetic acid molecular weight (60.05 mg/ mEq);
W = mass of sample (g) or sample volume (mL).
1000 = factor relating mg to grams (mg/g)
(1/10 = 100/1000)

Determination of colour: Colour was determined using Ovibond tintometre colorimeter model F. The cuvette used was first rinsed with ethanol. The samples were then poured into the cuvette and placed in the machine. The machine was then used to read the yellow and red parameters and the results were recorded.

Determination of phenol concentration: Phenol content was determined for the vinegar samples using the Folin-Ciocalteu method according to the the method described by [29]. Briefly, 1mL of the extract solution of each of the vinegar samples was mixed with 2.5mLs of 10% (w/v) Folin-Ciocalteu reagent for 5mins. After which 2.0mLs of 75% sodium carbonate (Na_2Co_3) was subsequently added to the mixture and incubated at 50°c for 10min with intermittent agitation. Thereafter, the sample was cooled and the absorbence was measured using UV spectrophhotometre (Shimazu, UV-1800) at 765nm against a reagent blank without extract.

Determination of Brix value: The refractometric method was used to analyse the degree of brix of the samples. Two drops of each of the samples was applied to the lower prism. The prism chamber was locked. A light source was applied and the shadow was viewed through the telescope ensuring that the eye was at the centre of the eye piece. The shadow edge as aligned for sharp intersection of the cross hairs. The degree brix was read from the Brix scale ensuring that the temperature was noted.

Determination of sensory attributes: The evaluation was conducted out-door in the morning before breakfast under natural light; using a 20 member trained panelists drawn from the public. The panelists were given a consent form and were intimated on their duties. The panelists were required to observe the sample, taste and score. Then rinse their mouth with water before tasting another sample.

The samples was analyzed based on the following parameters of appearance, aroma, mouth feel, taste, thickness and overall acceptability using a nine-point Hedonic scale of 9 = liked extremely down to I = disliked extremely [30]. For the colour, the vinegar was presented in transparent glass bottles and aroma was accessed using a brown glass bottle to minimize any clue coming from appearance [31].

3. Results

The result on Table 1 presents the different pH values of the vinegar samples at different days in the fermentation. The 7th day showed a pH of 8.3, 7.5 and 8.0 for Vin A,B,C respectively. On the14th day, there was a slight decrease in the pH of the vinegar as the pH dropped to 7.5, 7.3, 7.2 in Vin A,B,C respectively. On the 21st day of the fermentation, the pH dropped further to 7.2, 7.0, 7.5 in Vin A,B,C respectively as can be seen in Table 1. On the 35th day, the pH reduced to 6.8, 6.5 and 7.0 in Vin A,B,C respectively. On the 49th day, the pH reduced to 6.2, 5.5 and 6.5 in Vin A,B,C respectively. On the 63rd day, the pH reduced to 5.8, 4.7 and 5.3 in Vin A,B,C respectively. On the 77th day, the pH reduced to 4.5, 3.7 and 4.0 in Vin A,B,C respectively. On the 90th day, the pH reduced to 3.46, 3.22 and 3.32 in Vin A,B,C respectively. These changes in the pH could be a result of the fermentation process and a function of an increase in the acidity composition formed in the samples as the fermentation period increases. The result on Table 2 shows the different titrable acidity values of the vinegar samples at different days in the fermentation. The 7th day showed a titrable acidity of 0.015%, 0.067% and 0.013% for Vin A,B,C respectively. On the14th day, there was a slight increase in the titrable acidity of the vinegar as the titrable acidity increased to 0.020%, 0.090%, 0.018% in Vin A,B,C respectively. On the 21st day of the fermentation, the titrable acidity increased to 0.050%, 0.150%, 0.030% in Vin A,B,C respectively as can be seen in Table 2. On the 35th day, the titrable acidity increased to 0.15%, 0.34% and 0.10% in Vin A,B,C respectively. On the 49th day, the titrable acidity increased to 0.50%, 0.74% and 0.30% in Vin A,B,C respectively. On the 63rd day, titrable acidity increased to 0.80%, 1.00% and 0.50% in Vin A,B,C respectively. On the 77th day, the titrable acidity increased to 1.08%, 1.50% and 1.00% in Vin A,B,C respectively. On the 90th day, the titrable acidity increased to 1.18, 1.94 and 1.17 in Vin A,B,C respectively. This change in the titrable acidity is gradual and could also be attributed to the conversion of the alcohols to acetic acid which is the major organic acid found in vinegars. Other organic acids can be formed and can also be said to play roles in increasing the titrable acidity of the vinegar. The result on Table 3 shows the different relative density values of the vinegar samples at different days in the fermentation. The relative density just like the titrable acidity also increased with increase in the fermentation period. The 7th day showed a relative density of 1.066, 1.066 and 1.068 for Vin A,B,C respectively. On the14th day, there was a slight increase in the titrable acidity of the vinegar as the relative density increased to 1.80, 1.080 and 1.082 in Vin A,B,C respectively. On the 21st day of the fermentation, the relative density increased to 1.095, 1.095 and 1.098 in Vin A,B,C respectively as can be seen in Table 3. On the 35th day, the relative density increased to 1.105, 1.102 and 1.108 in Vin A,B,C respectively. On the 49th day, the relative density increased to 1.102, 1.110 and 1.125 in Vin A,B,C respectively. On the 63rd day, relative density increased to 1.138, 1.126, 1.153 in Vin A,B,C respectively. On the 77th day, the relative density increased to 1.150, 1.130 and 1.160 in Vin A,B,C respectively. On the 90th day, the relative density increased to 1.200, 1.200 and 1.210. From the results shown in Table 1 - Table 3, it can be said that there is an increase in titrable acidity and relative density with increase in the fermentation period but a decrease in pH which is inversely proportional to the titrable acidity of the vinegar samples. Six colonies in total were selected from the culture plates with the vinegar because of the presence of the desires halo formation on the selected media used and designated as AABA1, AABB2, AABB3, AABC1, AABC2 AABC3**.** The cultural characteristics of the isolates are shown in Table 4. It showed that the isolates had similar cultural characteristics. From the results on Table 5, the isolates are seen to be gram negative rods and are motile. The results from Table 6 shows the organisms had similar biochemical properties of the isolates. The result on Table 7 shows the microbial isolation for quality assessment. It showed no growth of pathogenic organisms like *Escherichia coli*, Mould, Coliforms and no growth in the plates for total viable counts. The result on Table 8 shows the physicochemical properties of the locally and industrially produced vinegar. The pH content of the vinegars (Vin A, B, C, D) are 3.46, 3.22, 3.32, 3.22 respectively. The titrable acidity of the vinegars (Vin A, B, C, D) are 1.18, 1.94, 1.17, 5.14. The relative densities of the vinegars of the vinegar (Vin A, B, C, D) are 1.20, 1.20, 1.21, 1.22 respectively. The colour was measured in values which are 7.3.4°, 7.3.5°, 9.5.9° and 9.3.5° for Vin A,B, C and D respectively. The total phenol concentration of the samples includes 7.0, 2.0, 112.00, and 6.70µg/ml for Vin A, B, C and D respectively. The brix value of the samples include 9.90, 14.80, 27.80, and 1.60°brix for Vin A, B, C and D respectively. The sensory parameters evaluated include Colour, Taste, Aroma, mouth feel, thickness and overall acceptability. The colour of Vin D scored the highest with 7.70 followed by Vin A, B and C with 7.20, 7.1 and 5.3 respectively as can be seen in Table 9. The taste of Vin C scored the highest with 7.0 followed by Vin D, C and B with 6.7, 6.5 and 5.8 respectively. The aroma of Vin A and B were highly appreciated and hence scored the highest with a score of 8.0 followed by Vin D with 7.9 and Vin C with 7.5.The mouth feel of Vin C scored the highest with 6.7followed by Vin A, D and B with 6.4, 6.2 and 5.8 respectively. The thickness of Vin C was highly appreciated with the score of 7.9 followed by Vin A,B and D with 7.5, 6.9 and 6.8 respectively. General acceptability of Vin A scored the highest with 8.1 followed by Vin D,C and B with 8.1, 7.6, 7.3.

Table 1. The changes in the pH values of the vinegar in different days

Days	Vin A	Vin B	Vin C
7	7.3±0.3	7.5±1.0	8.0±0.02
14	7.8±0.4	7.3±1.2	7.7±0.5
21	7.2±0.90	7.0±0.3	7.5±1.0
35	6.8±0.20	6.5±0.42	7.0±0.72
49	6.2±0.29	5.5±0.56	6.5±0.24
63	5.8±1.0	4.7±0.89	5.3±0.50
77	4.5±0.3	3.7±0.9	4.0±0.39
90	3.46±0.10	3.22±0.21	3.32±0.42

Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and orange

Table 2. The changes in the titrable acidity values of the vinegar in different days

Days	Vin A (%)	Vin B(%)	Vin C(%)
7	0.015±0.002	0.067±0.44	0.013±1.0
14	0.018±0.050	0.090±0.59	0.018±0.66
21	0.050±0.100	0.150±0.38	0.030±0.22
35	0.100±0.120	0.340±0.77	0.100±0.23
49	0.500±0.690	0.740±0.33	0.300±0.33
63	0.800±0.200	1.000±0.29	0.500±0.56
77	1.080±0.330	1.500±0.22	1.130±0.66
90	1.180±0.450	1.940±0.47	1.170±1.20

Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and oranges

Table 3. The changes in the relative density values of the vinegar in different days

Days	Vin A	Vin B	Vin C
7	1.066±0.66	1.066±0.11	1.068±035
14	1.080±1.00	1.080±1.0	1.082±0.94
21	1.095±0.23	1.095±0.67	1.098±0.27
35	1.105±0.22	1.102±0.22	1.108±0.33
49	1.120±0.34	1.110±0.45	1.125±0.45
63	1.130±0.22	1.126±0.23	1.153±0.72
77	1.150±0.22	1.130±0.90	1.160±0.11
90	1.200±0.33	1.200±0.22	1.210±0.50

Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and orange

Table 4. Cultural characteristics of the isolates

Isolates	Shape	Colony colour	Opacity	Elevation	Surface
AAB A1	Circular	Off white	Pale	Slightly raised	Smooth
AAB B2	Irregular circular	Milky white	Pale	Convex	Smooth
AAB B3	Circular	Off white	Opaque	Raised	Smooth
AAB C4	Circular	Creamy white	Opaque	Slightly raised	Smooth
AAB C5	Irregular circular	Off white	Pale	Convex	Smooth
AAB C6	Irregular circular	Off white	Opaque	Raised	Smooth

Table 5. Morphology and staining properties of the isolates

Isolates	Gram stain	Colour	Shape	Motility
AAB A1	Negative	Pink	Small rods	+
AAB B2	Negative	Pink	Rods	+
AAB B3	Negative	Pink	Rods, roundish	+
AAB C4	Negative	Pink	Rods	+
AAB C5	Negative	Pink	Rods	+
AAB C6	Negative	Pink	Rods	+

Table 6. Biochemical Characteristics of the Isolates

Isolates	Indole	Methyl red	Voges-Proskauer	Motility test	Catalase test	Indole test	Oxidase test	Sucrose	Maltose	Mannitol	Lactose	Organisms
AAB A1	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*
AAB B2	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*
AAB B3	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*
AAB C4	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*
AAB C5	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*
AAB C6	-	+	-	-	+	-	-	AG	-	-	-	*Acetobacter sp*

Table 7. Microbiology Quality of the Vinegar Samples

Parameters	Vin A (cfu/ml)	Vin B (cfu/ml)	Vin C (cfu/ml)	Vin D (cfu/ml)
Total viable count	0	0	0	0
Mould	0	0	0	0
Coliform count	0	0	0	0

Table 8. Physicochemical analysis of the produced vinegar

Sample	pH	Titrable acidity (%)	Relative density	Colour	Phenol (µg/ml)	Sugar content (°Brix)
Vin A	3.46 ±0.10	1.18 ±0.54	1.20 ±0.11	7.3.4°	7.00±0.00	9.90±0.05
Vin B	3.22 ±0.21	1.94 ±0.72	1.20 ±0.60	7.3.5°	2.00±0.500	14.80±0.00
Vin C	3.32 ±0.37	1.17 ±0.12	1.21 ±0.23	9.5.9°	112.00±0.25	27.80±0.00
Vin D	3.22 ±0.05	5.14 ±0.13	1.22 ±0.30	9.3.5°	6.70±0.20	1.60±0.05

Vin A = vinegar from combination of lemon, lime, orange, grape (all with the peel)
Vin B= vinegar from combination of green and red apple with the peels bought from Awka
Vin C= vinegar from vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother.

Table 9. Sensory evaluation of the vinegar samples

Parameters	Vin A	Vin B	Vin C	Vin D
Colour	7.2±1.90	7.1±1.65	5.3±1.38	7.70±1.2
Taste	6.50±0.91	5.8±1.51	7.0±1.66	6.7±1.3
Aroma	8.0±0.91	8.0±1.21	7.5±1.16	7.9±0.5
Mouth feel	6.4±1.91	5.8±1.2	6.7±1.5	6.2±1.6
Thickness	7.5±1.2	6.8±1.0	7.9±1.2	6.9±0.8
Overall acceptability	8.1±1.3	7.3±1.8	7.6±1.5	8.0±1.8

Vin A = combination of lemon, lime, orange, grape (all with the peel)
Vin B= combination of green and red apple bought from Awka
Vin C=vinegar from pawpaw, jackfruit, pineapple with peel and oranges
Vin D= Bragg (organic) raw unfiltered apple cider vinegar with mother.

4. Discussion

Vinegar is a fermented drink that is produced from both alcoholic and acetic acid fermentation of sugary substrates or raw materials. pH changes due to amount of organic or total acids present in the vinegar [32]. From the results of Table 1, it can be seen that the there is a decrease in the pH of the samples with increase in the fermentation days. This result is similar to the work of Onuorah *et al* [33] who also recorded an increase in the pH of the vinegars produced with an increase in fermentation time. There is no significant difference in pH among the vinegars analysed. This result in Table 4 agrees with the work of Jamaludin *et al* [34]. The pH value in this work is similar to the result of Onuorah *et al*., [33] who also got a pH of 3.5 in the 4th week of fermentation in the production of vinegar from oil-palmwine. The lower the pH value shows the higher acidity. From the results in Table 4, Vin D and B had the same pH value; this could be as a result of the presence of common raw materials which is apple hence it is safe to say that vinegars produced from common raw materials will give a common pH. Raw materials play a big role in the pH content of the vinegar samples. From the result in Table 2, it shows that there is a gradual increase in the titrable acidity of the samples with increase in the fermentation time. This result is similar to the work of Onuorah *et al* [33] who also recorded an increase in the titrable acidity of the vinegars produced with an increase in fermentation time The titrable acidity of produced vinegars and the industrial vinegar (Vin A,B,C and D) is 1.18%, 1.94% 1.17% and 5.13 % respectively as can be seen in Table 4. None of the locally produced vinegar reached an acid level of 5%. This could be because fermentation time and also the fermentation methods were

probably not optimal. However, there is potential for better vinegar production, if the process is improved. The reason of generation of acidity in vinegar is because of the different organic acids that are produced during acetic fermentation process, which is also due to breakdown of those sugars which are capable of fermentation, in addition to the presence of dissolved solid materials. There was a change in the relative density with increase in the fermentation days as can be seen in Table 3. There is an increase in the relative density with increase in the fermentation time. This is could be as a result of the changes undergone in the samples during the fermentation periods. The relative density of the produced vinegars and the industrial vinegar (Vin A, B, C and D) is 1.20, 1.20 1.21 and 1.22 respectively. This result is in keeping with vinegars produced from slow traditional methods [35]. This result is also similar to the work of Raichurkar and Dadagkhair [36] on custard apple vinegar

Acetobacter strains are the major acetic acid bacteria that are involved in vinegar production industrially [37]. The presence of the distinctive morphology on the selective media aided in the selection of the six acetic acid bacteria. The cultural characteristics are similar to the work of Kowser *et a*l [38] in the isolation of acetic acid bacteria from rotten papaya. Similarly Zahoor *et al*. [25] founds that the acetic acid bacteria colonies were smooth, small, medium, big and white, off white, pale, spherical, raised, convex, spheroid, star shaped, rough, crinkled and flat. The gram reaction of the isolates is negative and is rod like in shape and the isolates are seen to be motile. This is result is similar to the work of Kowser *et al* [38] in isolation of acetic acid bacteria from rotten papaya. Generally, the results of this work is similar to the findings Zahoor *et al*., [25] in isolation and characterization of *Acetobacter aceti* from indigenous source, Loganathan [39] in isolation of *Acetobacter diazotrophicus* fom *Eleusine coracana L*. Similar results were also obtained by Yamada *et al*. [40], Lisdiyanti *et al*. [41], Ashecraft *et al*. [42] who also isolated *Acetobacter spp*. and found that morphologically these are gram negative rods and Kadere *et al*. [9] found *Acetobacter aceti* was motility positive. The major isolates from the result is the acetic acid bacteria particularly from the genus *Acetobacter* and this is because they are capable of oxidizing sugars, sugar alcohols and alcohols to corresponding acids [43]. Acetic acid is the predominant organic acid produce from acetic acid bacteria [44] which is in line with this work because the vinegar produced contains significant quantity of acetic acid produced by acetic acid bacteria predominantly, *Acetobacter*. The isolation and cultivation of acetic acid bacteria has always been described as problematic, resulting in an underestimation of acetic acid bacteria diversity when culture dependent methods are applied. This is especially true in the isolation from a high acetic acid level source [45]. Additionally, a viable but not cultivable (VBNC) state has been described for acetic acid bacteria, mainly in oxygen privation conditions [46] hence it is necessary to apply culture independent methods in elucidating the acetic acid bacteria population.

The microbiological assessment to determine the safety of the vinegar for consumption showed that there were no coliforrms, mould, *Echerichia coli* and the total viable count was zero which entails that the vinegar produced and the industrial vinegar is safe for consumption. The absence of moulds rules out the presence of aflatoxin in the sample. The absence of these organisms could be as a result of proper safety techniques or the fact that the acidic composition of the vinegar creates an unfavorable environment for the organisms.

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 [47].

The colour of Vin D scored the highest among all the vinegar analysed as can be seen in Table 9. This could have been because it gave a brighter colour than the rest of the vinegar. This was shortly followed by Vin A and B. Vin C had a thick brownish colour and scored the lowest among all the vinegar analysed. According to Liu *et al*., [32], the vinegar color is influenced by various factors, including the color of raw materials, chemical reactions during preparation, pigment produced by chemical or enzymatic reactions during fermentation, and the addition of caramel colorants, etc. Vinegar is a known for its sour taste. In this analysis, the taste of Vin C was highly appreciable than the other vinegar analysed. This is shortly followed by Vin D, A and B. The aroma and flavor of vinegars impacting on consumer acceptance is influenced by the raw materials used, the compounds formed during the fermentation process, and the fermentation type used [48,49,50,51]. The mouth feel of Vin C scored the highest among all the vinegar analysed followed by Vin A,D and C. The thickness of the Vin C scored the highest followed by the Vin A, D and C. Overall acceptability was highest with Vin A. This shows that it has the highest consumer acceptance among of the vinegar evaluated. Soussou *et al* [52] reported that all volatile organic acid short chains affect the aciditiy, flavor and overall quality of the vinegar. There was no negative rancid flavor of vinegar and this was similar to the work of Kang *et al* [31] in the evaluation of commercial grape vinegars stored for a long time.

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