



Microbial Quality and Sensory Assessment of Vinegar from Date Palm and Apple Fruits: Implications for Consumer Preference

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Abstract	Article History
Studies have shown that variations in production methods, raw materials quality and storage conditions may affect the sensory attributes and microbial safety of vinegar produced from fruit extracts. This study was carried out to evaluate the sensory and microbial quality of vinegar produced from date palm and apple fruits, exploring the implications for consumer preference and acceptance. Yeast and acetic acid bacterium were isolated from spoilt fruits and characterized using appropriate microbiological techniques. Vinegar production was carried out using submerged fermentation of must extracted from <i>Phoenix dactylifera</i> (PD) and <i>Malus sylvestris</i> (MS) fruits. Instrumentation and <i>in vivo</i> techniques were employed for the determination of acetic acid content and toxicity of the fruit vinegar. The data generated from this study were analyzed at 95% confidence level using Analysis of Variance (ANOVA), and post-hoc analysis using Turkey's test. Yeast and acetic acid bacterium were isolated from spoilt fruits and characterized using appropriate microbiological techniques. Vinegar production was carried out using submerged fermentation of must extracted from <i>Phoenix dactylifera</i> (PD) and <i>Malus sylvestris</i> (MS) fruits. Standard plate technique was employed for the microbial qualities of the vinegar samples. The sensory evaluation and acceptability of the vinegar samples were carried out among validated participants and analyzed using 9-point hedonic scale. The data generated from this study were analyzed at 95% confidence level using Analysis of Variance (ANOVA), and post-hoc analysis using Turkey's test. There were variations in microbial loads of the vinegar produced from MS and PD using <i>Saccharomyces cerevisiae</i> strain SR 128 (CS 128) and <i>Acetobacter aceti</i> strain WI (AAWI), but these were statistically non-significant ($P > 0.05$) and conform to the stipulated standard. The samples were well rated in terms of colour, taste and odour, and generally acceptable. Therefore, the prepared vinegar samples from MS and PD had microbial qualities that conformed with the stipulated standard, preferred and acceptable, and the sample prepared from PD was slightly better. Keywords: Vinegar, Microbial, Sensory, <i>Saccharomyces</i>, <i>Acetobacter</i>	Received: 15 Apr 2025 Accepted: 05 May 2025 Published: 10 May 2025  Scan QR code to view* License: CC BY 4.0*  Open Access article.
How to cite this paper: Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025). Microbial Quality and Sensory Assessment of Vinegar from Date Palm and Apple Fruits: Implications for Consumer Preference. <i>IPS Journal of Nutrition and Food Science</i>, 4(2), 410–417. https://doi.org/10.54117/ijnfs.v4i2.100.	

1. Introduction

Vinegar is a condiment produced from alcoholic fermentation followed by the acetification of glucose in fruits such as apples, pears, grapes, molasses, berries, honeys, and many other food rich in carbohydrates (Budak *et al.*, 2014). This process occurs in two stages, in which the first stage involves the conversion of sugar to ethanol by *Saccharomyces cerevisiae*, followed by the oxidation of ethanol into acetic acid under aerobic conditions (Hutchinson *et al.*, 2019). *Acetobacter acetii* cultures under aerobic conditions, incompletely oxidise alcohol produced by fermenting sugar to acetic acid, and eventually, vinegar will be synthesized. Generally, vinegar has been used as a therapeutic agent for tackling obesity, laryngitis, fever, swelling, and stomach ache since the 18th century (Bray, 2014).

In the food industries, vinegar is widely used for pickling vegetables and fruits, in condiments such as mayonnaises, ketchups, and salad dressings, and has traditionally been used as food seasoning and preservation ingredient (Saha and Benerjee, 2013). Different types of vinegar are made from different raw materials used in the fermentation for instance; rice vinegar is obtained from the acetous fermentation of sugars derived from rice; grain vinegar is derived from sorghum, wheat, or other grains; while fruit vinegar is synthesized from fruits such as grapes or apples. There are many other types of vinegar such as malt, wine, apple cider, and balsamic vinegar (Chen *et al.*, 2016).

Research has revealed that sensory evaluation of vinegar is paramount to its utility and acceptability by the final consumers (Sharma *et al.*, 2018). Acetic acid is the predominant flavouring and antimicrobial component in vinegar. The importance of acetic acid as a direct food additive or more recently as a food processing aid, to decontaminate food before distribution and consumption has been documented. Vinegar is a condiment that impacts the sensory properties of food. Not only this, it acts as a preservative due to its acetic acid content and thus, lowers the pH of food and hence, helps in preservation (Alboronoz, 2012). The wide diversity of products containing vinegar (sauces, ketchup, mayonnaise, etc.) testifies its usefulness and the current fall in wine consumption has favoured an increase in vinegar production.

Research has shown that pathogenic microorganisms are rarely isolated from vinegar due to the antimicrobial activity of acetic acid (Sharma *et al.*, 2018). The microbial evaluation of vinegar has revealed mostly acetic acid bacteria, which can only be isolated using a selective medium containing all the required nutrients for proliferation. A suitable microbiological medium such as Glucose Yeast extract Calcium carbonate (GYC) are essential for the isolation acetic acid bacteria due to fastidious nature of the bacteria.

Several researchers have worked on the sensory and microbial quality assessment of vinegar produced from natural fruits such as Alboronoz (2012), Sharma *et al.* (2018), Mathew *et al.* (2019) but few information had been documented on the sensory and microbial quality assessment of vinegar produced from *Phoenix dactylifera* and *Malus sylvestris* fruits. Hence,

the aim of this study is to evaluate the sensory and microbial quality assessment of vinegar produced from *Phoenix dactylifera* and *Malus sylvestris* fruits.

2. Materials and Methods

Isolation and Characterization of *Saccharomyces* species from Spoilt Fruit Samples

Sample collection

This was done using a method described by Iheukwumere *et al.* (2025a). Spoilt *Musa paradisicum* (Banana) and *Citrus aurantium* (orange) fruits were collected from different points in Nkwo Oba market, Idemili South LGA, Anambra State. The fruits were detected through sight and nasal perception, followed by carefully and selectively picking the detected fruits into polyethene bags. The polythene bags were appropriately labelled and transported immediately to the laboratory for further analysis.

Sample preparation

This was done using a method described by Iheukwumere *et al.* (2025). The fruit samples were thoroughly washed using distilled water and their ectocarps were appropriately peeled using stainless chicken knife. The peeled fruits were pulverized using electric blender (SMX425/Japan). This was serially diluted (1:10) using 250 mL conical flask (Pyrex) in the capacity of 10 g of the fruit sample to make up 200 mL of the sample solution. The solution was thorough shaken, stoppered and kept for further analysis.

Isolation of yeast

This was done using a method described by Iheukwumere *et al.* (2022). The Sabouraud Dextrose Agar (SDA) and Yeast Extract Agar (YEA) were prepared according to the manufacturer's direction. The prepared media were autoclaved at standard conditions (121°C 15PSI at 15 min). The media were aseptically poured in Petri dishes and allowed to solidify. An aliquot of 0.1 mL of the prepared sample was aseptically spread on the surfaces of the agar poured plates and incubated at an inverted position at 35±2°C for 24 hours.

Characterization of the yeast

The yeast isolate was characterized morphologically, biochemically, and molecularly using the method described in Cheesbrough (2010) and Iheukwumere *et al.* (2020). It was also physically examined, and its colour, shape, texture, elevation, and consistency were recorded.

Isolation of Acetic Acid Bacterium from Spoilt Fruit Samples

This was carried out using a method in Okpalla *et al.* (2012), Glucose-Yeast Extract Calcium Carbonate (GYC) agar prepared from glucose (10%), CaCO₃ (2%) and agar (1.5%). The re-constituted medium was autoclaved at standard conditions (121°C, 15 PSI at 115 min). The medium was aseptically distributed into different Petri dishes and allowed to solidify. An aliquot of 0.1 mL of the prepared sample from the spoilt fruits was aseptically spread on the surfaces of the prepared agar medium and these were incubated in the inverted position at room temperature (30±2°C) for 48 h. Colonies with large clear zones around them were subcultured.

Characterization of the Bacterial Isolate

The pure isolates will be characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2018a). The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates will be carried out as described in Iheukwumere *et al.* (2018b). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement will also be carried out using the procedure described by Cheesbrough (2010), Iheukwumere *et al.* (2018c). The presence or absence of capsule will also be carried out as described by Iheukwumere *et al.* (2018d). The presence or absence of flagellum will be determined by carrying out motility test as described by Cheesbrough (2010). The capability of the isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55 % NaCl and to utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, sacharose, glucose trehalose, lactose, starch, inulin, salicin, hiparate) and also the haemolytic activity of the isolates were done using the methods described by Cheesbrough (2010) and Iheukwumere *et al.* (2018a). The molecular characterization involved DNA extraction, authentication, amplification and sequencing of the amplicons (Iheukwumere *et al.*, 2018b).

Vinegar Production

Collection and preparation of fruit samples for production of vinegar

Phoenix dactylifera (commonly known as Date) and *Malus sylvestris* (commonly known as English Apple) fruits were bought from Eke Awka Market, Anambra State. The fruit samples were thoroughly washed using distilled water and their ectocarps were thoroughly peeled. These were separately pulverized using electric blender (SMX 425/Japan). The pulverized fruits were extracted using distilled water. The solutions were then filtered using muslin cloth.

Production of alcohol

Here, 400 mL of the fruit extract was dispensed each into 500 mL conical flask (Pyrex). The extracts were sterilized using an Autoclave at standard conditions (121°C, 15 PSI at 115 min). The sterilized extracts were allowed to cool. The extracts were each inoculated *Saccharomyces cerevisiae* strain and allowed for 28 days with manually daily shaking at 30±2°C. After the fermentation, the alcohol was decanted and poured into sterile 2000 mL bottle and allowed open for 2 days.

Alcohol tolerance test

The ability of the acetic acid bacterium to grow in the presence of alcohol was carried out using the method described in the study published by Tharinee *et al.* (2015). The tested isolate was grown in yeast extract agar (0.50% yeast extract, 2% agar) supplemented with 2%, 4%, 6%, 8%, and 10% (v/v) absolute ethanol. The above procedure was then modified by growing the isolate in Glucose-Yeast Extract Calcium Carbonate (GYC) broth/agar supplemented with 2%, 4%, 6%, 8%, and 10% (v/v) absolute ethanol.

Vinegar production

The colonies of *Acetobacter aceti* strain was aseptically transferred into the container containing the alcohol. The bottles were thereafter covered with sac cloth to prevent the

entry of insect. The set-up was allowed for 28 days at room temperature (30±2°C). At the end of the fermentation period, a thick film known as mother of vinegar had covered the surface of the vinegar and was carefully scooped out to avoid contamination. The vinegar was thereafter filtered.

Microbial Analyses of the Vinegar

The microbial analyses of the vinegar was carried out using standard plate count technique as described in Cheesbrough (2010) with slight modification in the choice of media used. The total spore counts was carried out by growing the heated samples on Nutrient Agar (BIOTECH) at 35±2°C for 24 h. The total *Bacillus cereus* counts was carried out by growing the heated samples on Mannitol Nutrient Agar (MNA) at 35±2°C for 24 – 48 h. The total mesophilic aerobic bacterial counts was carried out by growing the samples on nutrient agar (BIOTECH) at 35±2°C for 24 h. The total acetic acid bacterial count was carried out by growing the samples at GYC agar at room temperature (30±2°C) for 24 h. The total lactic acid bacterial counts was carried out by growing the samples on Demann Rogosa Sharpe (MRS) agar at 30±2°C for 48 h. The total yeast and mold counts were carried out by growing the samples on Sabouraud Dextrose Agar (SDA) at 35±2°C and 30±2°C, respectively for 24 hours and 5 days respectively.

Sensory Evaluation of the Prepared Vinegar

Sensory Evaluation: In house consumer-oriented test was conducted to determine product acceptability using a scoring test with the aid of a 9-point hedonic scale with little modification in the studies published by Adedokun *et al.* (2013). The sensory characteristics of the vinegar samples such as colour, odour, taste and general acceptability were examined by the team of twenty (20) validated panellists which was drawn from microbiology students of Chukwemeka Odumegwu Ojukwu University, Uli. The panellists were validated in such a way that they were able to detect little perceptible changes in the sensory attributes mentioned. Each panellist was asked to score each coded sample based on a nine point hedonic scale (like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much, dislike extremely).

Statistical Analysis

The data generated from this study were analyzed at 95% confidence level using Analysis of Variance (ANOVA), and post-hoc analysis using Turkey's test (Iheukwumere *et al.*, 2022a).

3. Results

Characterization of the Yeast Isolate and Acetic Acid Bacteria Strains

The yeast isolate (XI) showed characteristic features of yeast such as cream white colonies on Sabouraud Dextrose Agar (SDA) plate, smooth surface, spherical morphology and utilization of glucose and sucrose. The yeast was also resistant to cycloheximides as shown in Table 1. The acetic acid bacterium (AI) showed cream to yellow colonies on glucose yeast extract calcium carbonate agar (GYA). The isolate was also Gram negative rod, motile, catalase, methyl red and Voges Prokauer positive, but indole, oxidase and citrate

negative as shown in Table 2. The quality and nature of the extracted nucleic acid revealed 260/280. Hence, Deoxyribonucleic acid (DNA) as shown in Table 3. The molecular identities of the isolates revealed 100% query cover and 100% identities. This revealed that sample 1D AI was *Acetobacter aceti* strain WI (AAWI) whereas sample ID XI was *Saccharomyces cerevisiae* strain Ysr128 (SC 128) as shown in Table 4

Alcohol Tolerance Potential of the Test Isolate

The study revealed that the test isolate was able to grow in the presence of 10% absolute alcohol. There was significant ($P < 0.05$) number of colonies of acetic acid bacteria in 10% absolute alcohol level in both yeast extract agar (YEA) and glucose-Yeast extract calcium carbonate agar (GYA). The number of colonies slightly decreased as the concentration of alcohol increased as shown in Table 5 but the decrease was statistically non-significant ($P > 0.05$).

Microbial Qualities of the Vinegar Samples

The study revealed count values for total mesophilic aerobic bacterial counts (TMABC), total acetic acid bacterial counts (TABC) and total yeast counts (TYC) for the three vinegar samples. The TMABC was significantly ($P < 0.05$) higher in vinegar bought from the supermarket (VS) whereas the

vinegar prepared from apples (VA) and dates (VD) non-significantly ($P > 0.05$) showed slight variations in their TMABC as shown in Table 6. There was also slight variations in TABC of VA and VD and these were non-significantly ($P > 0.05$) higher than that of VS.

There were variations in TYC, and this was non-significantly ($P > 0.05$) most in VA. There was no total spore counts (TSC), total *Bacillus cereus* counts (TBCC), total lactic acid bacterial counts (TLBC) and total mold counts (TMC) detected in VA and VD; TSC, TBC, TLBC, and TMB were within the stipulation limit of National Industrial Standard (NIS).

Sensory Evaluation and Acceptability of the Vinegar Samples

The study on the sensory evaluation and acceptability of the vinegar samples are shown in Table 7. The study revealed that the colour of the vinegar samples were like moderately in the 9-point hedonic scale. The taste of the vinegar samples were within neither like nor dislike points, and the odour was within like slightly in the point scale. The general acceptability of the vinegar samples was seen within like very much in the 9-point hedonic scale.

Table 1: Morphological and biochemical characteristics of the yeast isolates

Parameter	X1	X2
Appearance on GYA	Cream white colonies	Cream white colonies
Surface	Smooth	Smooth
Margin	Circular	Circular
Elevation	Convex	Convex
Shape	Spherical	Spherical
Bud	Present	Present
Ascospore	Present	Present
Glucose	+	+
Sucrose	+	+
Maltose	+	+
Gelactose	+	+
Raffinose	+	+
Mannitol	—	—
Lactose	—	—
Xylose	—	—
Cyclohexide	Resistance	Resistance
Suspected yeast	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i>

Table 2: Morphological and biochemical characteristics of the acetic acid bacterium

Parameter	A1	A2
Appearance on GYA	Cream to yellow colour	Cream to yellow colour
Surface	Smooth	Smooth
Elevation	Convex	Convex
Opacity	Opaque	Opaque
Shape	Rod	Rod
Arrangement	Clustered	Clustered
Gram Reaction	—	—
Motility	+	+
Indole	—	—
Citrate	—	—
Catalase	+	+
Methyl red	+	+
Voges Proskauer	+	+
Oxidase	—	—
Glucose	+	+
Sucrose	+	+
Mannitol	+	+
Bacterium	<i>Acetobacter</i> species	<i>Acetobacter</i> species

Table 3: Quality and nature of the extracted nucleic acid

Sample ID	Nucleic acid(µg/mL)	260 nm	280 nm	260/280
A1	120.20	3.412	1.875	1.82
X1	102.10	3.104	1.687	1.84

Table 4: Molecular identities of the isolates

Parameter	A1	X1
Max Score	2676	6205
Total Score	2676	6604
Query Cover (%)	100	100
E-Value	0.0	0.0
Identity (%)	100	100
Accession Length	1449	224595
Accession Number	1ICC662508.1	CP036471.1
Description	<i>Acetobacter aceti</i> strain W2 (AAW1) 16S rRNA gene partial sequence	<i>Saccharomyces cerevisiae</i> strain Ysr128 (SC128) chromosome 1, complement sequence

Table 5: Alcohol tolerance of the test isolate

Alcoholic Content (%)	Yeast Extract Agar		Glucose-Yeast Calcium Carbonate	Extract
	Count (CFU/mL)	Log CFU/mL	Count (CFU/m)L	Log CFU/mL
2.0	5.10X10 ²	2.71	6.40X10 ²	2.81
4.0	4.70X10 ²	2.67	6.10X10 ²	2.79
6.0	4.30X10 ²	2.63	5.70X10 ²	2.76
8.0	4.10X10 ²	2.61	5.40X10 ²	2.73
10.0	3.80X10 ²	2.58	5.10X10 ²	2.71

Table 6: Microbial qualities of the vinegar samples

Count	VA	VD	VS
TMABC (CFU/mL/LogCFU/mL)	5.40X10 ² (2.73)	5.10X10 ² (2.71)	9.20X10 ² (2.96)
TBCC(CFU/mL/LogCFU/mL)	0(0)	(0)	0.60X10 ² (1.78)
TSC (CFU/mL/LogCFU/mL)	0(0)	0(0)	2.20X10 ²
TABC(CFU/mL/LogCFU/mL)	7.60X10 ² (2.88)	7.90X10 ² (2.90)	6.80X10 ² (2.83)
TLBC(CFU/mL/LogCFU/mL)	0(0)	0(0)	1.20X10 ² (2.08)
TYC(CFU/mL/LogCFU/mL)	2.60X10 ² (2.41)	2.10X10 ² (2.32)	6.80X10 ² (2.226)
TMC(CFU/mL/LogCFU/mL)	0(0)	0(0)	0.30X10 ² (1.48)

Table 7: Sensory parameters and acceptability of the vinegar samples

Parameter	VA	VD	VS
Colour	0.74±0.01	0.71±0.01	0.76±0.01
Taste	0.51±0.001	0.52±0.01	0.56±0.01
Odour	0.64±0.01	0.62±0.01	0.61±0.01
General Acceptability	0.82±0.01	0.84±0.01	0.84±0.1

Discussion

The presence of *Saccharomyces cerevisiae* strain Ysr128 (SC 128) from the spoilt banana samples corroborated with the findings of Jayamma *et al.* (2022). The characteristic features of the yeast isolate such as cream white appearance on Sabouraud Dextrose Agar (SDA), resistant to cycloheximide, utilization of sugars were also reported by many researchers (Amanul *et al.*, 2017; Kechkar *et al.*, 2019; Kumari *et al.*, 2019; Jayamma *et al.*, 2022; Petruzzello *et al.*, 2023). The presence of *Acetobacter aceti* strain w1 (AAWI) in banana juice supported the findings of Srivastava and Rani (2019) and Wang *et al.* (2022). The characteristic features of *Acetobacter* revealed in this study corroborated with the findings of Afrifuzzaman *et al.* (2018), Quattara *et al.* (2018), Srivastara and Rani (2019) and Wang *et al.* (2022).

Vinegar is a liquid fermented product that contains fruits juice as the main ingredients which contain many functional compounds such as organic acids, vitamins, minerals, amino acids and phytochemicals such as phenolics, flavonoids, tannins and other phytochemicals. Similar reported was stated by Hamidalu (2014). In the present study, the production of vinegar from *Malus sylvestris* (green apple) and *Phoenix dactylifera* (date) agree with the findings of Kechka *et al.* (2019). Other researchers (Quattera *et al.*, 2018; Srivastara and Rani, 2019; Wang *et al.*, 2022; Safrida *et al.*, 2023) produce vinegar from various plants. The maximum acetic acid using green apple agrees with water Klawplyapamornkun *et al.* (2015) and Quattara *et al.* (2018) produced from fruits and mango juice, respectively. Vinegar produced from date fruits gave 5.2% of acetic acid and this was more than acetic acid produced from many other fruits.

Microbial counts recorded from the vinegar samples in the present study conform with the stipulation of National Industrial Standard (NIS). The mesophilic aerobic bacterial counts (TMABC), Acetic Acid bacterial counts (TABC) and yeast counts (TYC) recorded in this study conform with the NIS standard. The prepared vinegar samples recorded no count for *Bacillus cereus* (TBCC), Lactic acid bacterial count (TLBC) and mold count (TMC) and this was not in line with the findings of other researchers (Sengun *et al.*, 2020; Singh and Garg, 2022).

Sensory quality is an important indicator of overall quality and health safety of food products. The sensory characteristics of the study conform to the good quality and safety of the vinegar samples. Similar findings were reported by Morhtar *et al.* (2016) and Akarca *et al.* (2020).

Conclusion

The study has shown that the prepared vinegar samples from *Malus sylvestris* (MS/Apple) and *Phoenix dactylifera* (PD/Date) fruits had microbial qualities that conformed with the stipulated standard, preferred and acceptable, and the sample prepared from PD was slightly better.

Acknowledgments: We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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