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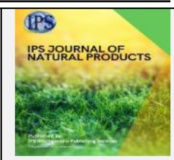

Date Palm and Banana Fruit Vinegar: Safety Evaluation of Acetic Acid Content and Toxicity

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Abstract

Vinegar is widely regarded as a safe food product; however, its specific safety profile, including acetic acid concentration and potential toxicological effects, can vary significantly based on raw materials and production methods. This study conducted a comprehensive safety evaluation of vinegar produced from *Phoenix dactylifera* (date palm) and *Musa* spp. (banana) by assessing its acetic acid content and *in vivo* toxicity. Yeast (*Saccharomyces cerevisiae* strain SR 128) and acetic acid bacteria (*Acetobacter aceti* strain WI) were isolated and used for submerged fermentation of fruit musts. Acetic acid content was measured using instrumental analysis, while toxicity was evaluated through a 28-day oral administration study in a rodent model, monitoring body weight, organ weights, and biochemical indicators of organ function. Statistical analysis was performed using Analysis of Variance (ANOVA) with Tukey's post-hoc test. The acetic acid concentrations were 4.10% for banana vinegar and 5.20% for date vinegar. The toxicity study revealed no significant adverse effects, with no statistically significant differences ($p > 0.05$) in body weight, organ weights, or organ function markers between treated and control groups. These findings indicate that vinegars from *Phoenix dactylifera* and *Musa* spp. contain acceptable acetic acid levels and demonstrate no evidence of toxicity in the tested model, supporting their safety for human consumption. The date palm vinegar exhibited a marginally higher acetic acid content, suggesting slightly superior fermentation efficiency.

Keywords: Vinegar, Toxicity, *Phoenix*, *Musa, Saccharomyces, Acetobacter*

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Introduction

Vinegar, a globally recognized condiment and functional food product, is produced through the acetic acid fermentation of ethanol derived from fermentable carbohydrate sources (Henke *et al.*, 2019; Iheukwumere *et al.,* 2025a; Dim *et al.,* 2025a). This bioconversion, mediated primarily by acetic acid bacteria, yields a product whose safety and functionality are intrinsically linked to its final acetic acid concentration and overall composition (Bhat *et al.*, 2014; Dim *et al.*, 2025b). While traditional substrates like grapes and apples dominate commercial production, there is growing interest in alternative fruits such as dates (*Phoenix dactylifera*) and bananas (*Musa* spp.) for vinegar fermentation, owing to their distinctive nutritional profiles and potential health benefits (Cusano *et al.*, 2020; Singh *et al.*, 2016; Amadi *et al.*, 2017; Nwike *et al.*, 2017). However, the safety parameters of vinegars derived from these unconventional substrates remain insufficiently characterized.

The production of fruit vinegar follows a two-stage biological process involving alcoholic fermentation by yeasts such as *Saccharomyces cerevisiae*, succeeded by acetification through bacterial action, typically by *Acetobacter* species (Matthew *et al.*, 2019; Ejike *et al.*, 2017; Ekechukwu *et al.*, 2025a). The resulting low pH (generally below 3.5) imparts antimicrobial properties but also necessitates careful evaluation of potential health risks, including dental erosion and mucosal irritation associated with excessive acidity or consumption (Gambon *et al.*, 2012; Loke *et al.*, 2016; Iheukwumere *et al.*, 2025b). Despite vinegar's general

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recognition as safe (GRAS) for culinary use, specific safety assessments are crucial for novel formulations, as substrate composition can significantly influence the fermentation dynamics, final acidity, and the presence of co-fermented compounds that may affect toxicological outcomes (Johnston, 2009; Ozturk *et al.*, 2015; Obianom *et al*., 2024).

Date vinegar has been studied for its rich phenolic content and antioxidant potential, while banana vinegar represents a promising avenue for valorizing surplus or overripe fruit, contributing to both waste reduction and the development of value-added products (Siddeeg *et al.*, 2016; Vu *et al.*, 2018; Iheukwumere *et al*., 2022a). Nevertheless, comparative studies evaluating the safety of vinegars from these two substrates are notably absent from the literature. Existing research on vinegar toxicity often focuses on conventional types or high-concentration acetic acid, leaving a critical gap in the safety profiling of artisanal fruit vinegars (Anderson *et al.*, 2020; Dim *et al*., 2023c). Therefore, this study aims to conduct a comprehensive safety evaluation of vinegar produced from *Phoenix dactylifera* (date) and *Musa* spp. (banana).

Materials and Methods

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

Sample collection

Spoilt *Musa paradisicum* (Banana) and *Phoenix dactylifera* (date palm) fruits were collected from different points in Nkwo Oba market, Idemili South LGA, Anambra State. The fruits were detected through sight and nasal perception; this was followed by careful and selective picking of the detected fruits into polyethene bags. The polythene bags were appropriately labelled and transported immediately to the laboratory for further analysis.

Sample preparation

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 shaked, stoppered and kept for further analysis as described by Egbe *et al.* (2025a), Egbe *et al*. (2025b), Iheukwumere *et al*.(2025c), and Iheukwumere *et al*. (2025d).

Isolation of yeast

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 as described in a study published by (Egbe *et al*., 2025c; Iheukwumere *et al*., 2022b; Iheukwumere *et al*., 2025e; Ekesiobi *et al*., 2025).

Characterization of the yeast

The yeast isolate was characterized morphologically, biochemically, and molecularly using the method described in Cheesbrough (2010), Iheukwumere *et al.* (2020a), Iheukwumere *et al.* (2020b); Ekechukwu *et al.* (2025b).The yeast isolate was physically examined; the colour, the shape, texture, elevation and the consistency were examined and recorded.

Isolation of Acetic Acid Bacterium from Spoilt Fruit Samples

This was carried out using 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 on inverted position at room temperature (30±2◦C) for 48 h. Colonies with large clear zones around them were subcultured (Chude *et al*., 2020; Ekechukwu *et al*., 2025c; Ezedianafo *et al*., 2025a; Idigo *et al*., 2025a; Iheukwumere *et al*., 2025f).

Characterization of the Bacterial Isolate

The pure isolates will be characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2017a); Iheukwumere *et al.* (2018a); Iheukwumere *et al*. (2020c). The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates will be carried out as described in Iheukwumere *et al.* (2017b); Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2024). 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), Iheukwumere and Iheukwumere (2022a) and Iheukwumere *et al.* (2023a). The presence or absence of capsule will also be carried out as described by Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2017d), and Iheukwumere *et al.* (2022c). The presence or absence of flagellum will be determined by carrying out motility test as described by Cheesbrough (2010), Iheukwumere *et al.* (2023b), Ezedianafo *et al.* (2025b), Ike *et al.* (2025a). 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), Iheukwumere *et al.* (2018), Iheukwumere and Iheukwumere (2022c), Iheukwumere *et al.* (2022d). The molecular characterization involved DNA extraction, authentication, amplification and sequencing of the amplicons (Iheukwumere *et al*., 2017e; Okeke *et al*., 2017; Iheukwumere *et al*., 2022e; Iheukwumere and Iheukwumere, 2022d).

Vinegar Production

Collection and preparation of fruit samples for the production of vinegar

Phoenix dactylifera (commonly known as Date) and *Musa paradisicum* (commonly known as Banana) 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 (Iheukwumere *et al*., 2022f; Iheukwumere and Iheukwumere, 2022e; Ezedianafo *et al*., 2025c).

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 as described by (Ike *et al*., 2025b; Obiefuna *et al*., 2025b; Ugwu *et al*., 2025a).

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 as described in a study published by Idigo *et al*. (2025b), Iheukwumere *et al*. (2025g), Ike *et al*. (2025c) and Ugwu *et al*. (2025b).

Acetic Acid Assay of the Vinegar

This was carried out using the method described in the study published by Onuorah *et al*. (2016), Iheukwumere *et al.* (2025h), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ike *et al*. (2025d). The assay was carried out in every 7 days interval. Here, 5 mL of the vinegar were added into a 250 mL conical flask containing 20 mL distilled water (1:5 dilution), then 5 drops of phenolphthalein was added into the flask and mixed the content thoroughly. The mixture was titrated against 0.5 N sodium hydroxide (NaOH) until the appearance of pale pink colouration was observed in the flask. The volume of NaOH consumed during the percentage of acetic acid calculated using the formula below:

$$\text{Percentage (\%)Acetic} = \frac{\text{Mass of Acetic Acid}}{\text{Mass of Vinegar}} x100$$

Toxicity of the prepared Samples

Albino Wistar rats: The albino Wistar rats were purchased at animal house , Zoology Department, University of Nigeria , Nsukka (UNN). The rats were transported to the animal house at Department of Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University (NAU), Awka. The rats were critically examined for their weights and experimented for their suitability for the study. The rats were selected and grouped based on their weights and experimented design.

In vivo Study: A total of 96 albino Wistar rats were used for this study. The rats were grouped into 3 groups. Each group had 5 subgroups that contained 6 rats each. The rats were orally administered 0.1, 1.0, 2.0, 4.0 and 5.0 mL of the prepared samples except the group that was giving ordinary distilled water as normal control. The rats in each group were monitored for 21 days during which the acute toxicity was determined after 72 h, liver enzymes, Kidney (creatinine, urea) and heart (Lactate dehydogenase LDH) monitoring parameters and effects on the cells (histopathology study) were checked and recorded as described in the work published by Iheukwumere *et al.* (2018) and Nwobodo *et al.* (2018) Ezedianafo *et al*. (2025d), Idigo *et al*. (2025d), Idigo *et al.* (2025e).

Acute toxicity: The albino Wistar rats were monitored for 72 h for mortality cases as described in the work published by Iheukwumere *et al*. (2018), Obiefuna *et al*. (2025b); Ike *et al.* (2025d); Idigo *et al*. (2025f).

Body and Organ weights: The body weights of the experimented rats were checked and recorded weekly using electronic weighing balance (LXD200). Also the organs from the sacrified rats were also weighed and recorded as described in the work published by Nwobodo *et al*. (2018), Idigo *et al.* (2025g), Idigo *et al*. (2025h).

Liver function test

Aspartate aminotransferase (AST) activity: This was carried out as described by Nwobodo *et al*. (2018), Idigo *et al*. (2025i); Idigo *et al*. (2025j), Idigo *et al*. (2025k); Idigo *et al*., (2025l). The blood sample was centrifuged and the serum was collected and dispensed 0.1 ml into test tube (pyrex), 0.5 ml of phosphate was added and mixed thoroughly. This was incubated at 37^0C for 30 min. Then 2,4 – dinitrophenylhydrazine was added to the mixture, mixed thoroughly and allowed to stand for 20min. Sodium hydroxide was added to the solution, mixed and allowed to stand for 5 min after which the absorbance was read at 546n m. The procedure was repeated for the blank without the sample and that of the standard. The AST activity was determined by the calibration curve provided in the kit.

Alanine aminotransferase (ALT) activity: This was carried out as described by Nwobodo *et al*. (2018), Idigo *et al*., 2025m; Idigo *et al*., 2025n; Idigo *et al*., 2025o. The clotted blood sample was centrifuged and the serum was collected and dispensed 0.1ml into the test tube and this was followed by the addition of 0.5 ml of phosphate buffer. This was mixed

thoroughly and incubated at 37^{0}C (Idigo *et al*., 2025p; Idigo *et al*., 2025q).

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.*, 2017b, Idigo *et al*., 2025r; Iheukwumere *et al*., 2025h; Iheukwumere *et al*., 2025i; Idigo *et al*., 2025s, Idigo *et al*., 2025t, Manasseh *et al*., 2025).

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. 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 cerevisae* 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 ($P > 0.05$).

Acetic Acid Production during Vinegar Production

The study revealed significant production of acetic acid within 28 days production set-up. There was non-significant ($P > 0.05$) increase in percentage of acetic acid produced in every 7 days interval but the level of acetic acid was significant ($P < 0.05$) after 21 days and 28 days, respectively for vinegar produced from apples and dates (Table 6). The study also revealed that the level of acetic acid produced from dates was higher than that produced from apple but the variation was statistically non-significant ($P > 0.05$).

Toxicity of the Vinegar Samples

The study revealed that the prepared vinegar samples were safe for consumption. The body weights of the rats increased in every 7 days intervals (Table 7), but this increase was statistically non-significant ($P > 0.05$) when compared with the control rats although there was a mild deceleration on the body weights of the rats fed with sample VS. There was slight increase in the weights of liver, kidney, hearts, lungs, and spleen of the rats that fed on the vinegar samples (Table 8), and these increase was observed most among the rats fed with sample VS, but these slight increase was statistically non-significant ($P > 0.05$) when compared to the normal rats. Also, there was slight increase in the LDH, Urea, Creatinine, ALT, and AST among the rats fed with the vinegar samples (Table 9), and this increase was detected most among the rats fed with sample VS, but this slight increase was statistically non-significant ($P > 0.05$). It was also observed that the ratio of AST/ALT for rats fed with sample VS was above 1.52 whereas other values were 1.52 and below. The metal analysis revealed the presence of cadmium, chromium, copper, mercury, lead, zinc, cobalt, and sodium. The vinegar samples contained significant concentration of sodium but varied among the samples, and this was statistically non-significant ($P > 0.05$). The concentrations of the heavy metals mainly lead, mercury, chromium and cadmium were low, but detected most in most sample VS. Sample VD contained the lowest concentrations of the heavy metals (Table 10).

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	Saccharomyces cerevisae	Saccharomyces cerevisae

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	*Acetobacter* species	*Acetobacter* 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	*Acetobacter aceti* strain W2 (AAW1) 16S rRNA gene partial sequence	*Saccharomyces cerevisiae* strain Ysr128 (SC128) chromosome 1, complement sequence

Table 5: Alcohol tolerance of the test isolate

Alcoholic Content (%)	Yeast Extract Agar		Glucose-Yeast Extract Calcium Carbonate	
	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: Acetic acid production during vinegar production

Day	VB (%)	VD (%)
7	2.10	2.60
14	3.20	3.90
21	4.10	5.10
28	4.10	5.20

Table 7: Effects of vinegar samples on body weight of rats

Day	N(g)	VB(g)	VD(g)	VS(g)
0	123.17±1.33	123.48±1.21	123.96±1.36	123.67±1.19
7	129.62±1.77	128.31±1.47	128.81±1.17	127.55±1.51
14	139.42±1.17	137.52±1.33	137.74±1.61	133.44±1.22
21	144.14±1.27	141.72±1.21	142.78±1.31	137.14±1.51

Table 8: Effects of the vinegar samples on organ weight of rats

Organ	N(g)	VB(g)	VD(g)	VS(g)
Liver	6.30±0.01	6.42±0.01	6.410±0.01	6.47±0.01
Kidney	0.48±0.00	0.51±0.00	0.52±0.01	0.58±0.00
Hearts	0.40±0.00	0.40±0.00	0.41±0.00	0.49±0.00
Lungs	1.02±0.00	1.08±0.00	1.04±0.00	1.18±0.00
Spleen	1.08±0.01	1.14±0.01	1.11±0.01	1.10±0.01

Table 9: Effects of the vinegar samples on organ functions

Sample	Heart	Kidney		Liver		
	LDH(U/L)	Urea(mg/dL)	Creatinine(mg/dL)	ALT(U/L)	AST(U/L)	AST/ALT
N	12.812	7.946	0.390	17.120	25.680	1.50
VB	13.847	8.740	0.430	17.470	26.550	1.52
VD	13.492	8.141	0.410	17.210	26.000	1.51
VS	14.212	8.810	0.440	18.630	29.540	1.59

Table 10: Metal values of the vinegar samples

Parameter	VB	VD	VS
Cadmium (ppm)	0.077	0.122	0.367
Chromium (ppm)	0.049	0.019	0.092
Copper (ppm)	0.396	0.399	0.578
Sodium (ppm)	8.493	9.278	9.566
Mercury (ppm)	0.032	0.029	0.097
Lead (ppm)	0.073	0.019	0.866
Zinc (ppm)	0.195	0.275	0.428
Cobalt (ppm)	0.167	0.078	0.155

Discussion

This study provides a systematic safety evaluation of vinegar produced from *Phoenix dactylifera* (date) and *Musa paradisiaca* (banana) fruits, with a primary focus on toxicological parameters and acetic acid content. Vinegar is a fermented liquid rich in functional compounds, including organic acids, vitamins, minerals, amino acids, and bioactive phytochemicals (Hamidalu, 2014; Iheukwumere *et al*., 2025j). The successful production of vinegar from these substrates aligns with broader research on fruit-based fermentations (Tengberg, 2012; Cantadori *et al*., 2022; Habiba *et al*., 2024; Iheukwumere *et al*., 2025k) and extends the application to banana, a less commonly studied source.

Microbiological analysis confirmed the presence of standard fermentative microorganisms. The yeast isolate from date fruit exhibited characteristics matching *Saccharomyces cerevisiae*, consistent with findings from previous studies on date fermentation (Mohammed *et al*., 2021; Atitallah *et al*., 2021; Ahmad *et al*., 2021). The identification of *Saccharomyces cerevisiae* strain Ysr128 further corroborates the work of Ahmad *et al.* (2021) and Ugobogu *et al.* (2025). Similarly, the bacterial isolate from banana displayed profiles consistent with *Acetobacter* species, as reported in other fruit vinegar studies (Fatima and Mishra, 2015; Prisacaru and Oroian, 2018; Armi *et al*., 2023), and aligned specifically with reports of *Acetobacter aceti* strain w1 (Boonsupa *et al*., 2019).

The acetic acid content, a critical safety and quality indicator, reached 5.2% in the date vinegar, a yield exceeding those reported for several other fruits. This result is consistent with research on high-acid substrates such as green apple and mango juice (Klawplyapamornkun *et al*., 2015; Ouattara *et al*., 2018; Iheukwumere *et al*., 2025l). The acetic acid level in banana vinegar was also within acceptable and functional ranges for food-grade products.

Toxicological assessment revealed no adverse effects, confirming the safety of both vinegar samples for consumption. The normal, progressive increase in body and organ weights in test subjects indicates an absence of systemic toxicity from sub-chronic intake. Furthermore, the maintenance of normal serum levels for liver enzymes (ALT, AST), urea, and creatinine suggests no significant hepatotoxic or nephrotoxic effects, corroborating safety profiles documented in other vinegar studies (Chandrasekaran and Bahkali, 2013; Saha *et al*., 2023; Alsarayrah *et al*., 2023; Iheukwumere *et al*., 2025m).

Analysis of heavy metal content showed that concentrations of lead, cadmium, and other trace metals in both vinegars were within the permissible limits stipulated by the Nigerian Industrial Standard (NIS). This finding aligns with safety reports for other fruit vinegars (Cantadori *et al*., 2022; Choudhary *et al*., 2025; Iheukwumere *et al*., 2025n). While

these compliant values differ from the elevated concentrations reported by Morht *et al.* (2016), they were comparable to levels detected in a commercial vinegar control sample. The presence of essential elements like sodium within safe limits may contribute to the nutritional value of the vinegars without introducing health risks.

Conclusion

The study has shown that the prepared vinegar samples from *Musa paradisicum* (MP/Banana) and *Phoenix dactylifera* (PD/Date) fruits contained stipulated acetic acid levels, and they are safe for human consumption. The sample prepared from PD was slightly better.

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Ethical approval: All authors hereby declare that "Principles animal care" (NCARE with Ref No FPSRA/UNN/24/0113), certified on 24th November, 2024 at University of Nigeria, Nsukka, were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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

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