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Safety Evaluation of Vinegar from *Phoenix dactylifera* and *Malus sylvestris*: Toxicity and Acetic Acid Content

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Abstract

Vinegar is generally considered safe, but variations in production methods, acetic acid content and potential contaminants may impact its safety profile. This study was carried out to evaluate the safety of vinegar produced from *Phoenix dactylifera* (PD) and Malus sylvestris (MS) by assessing its toxicity and acetic acid content, providing essential information for consumers, manufacturers and regulatory agencies. 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 *Phoenix dactylifera* (PD) and *Malus sylvestris* (MS) fruits. Instrumentation and *in vivo* 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. Vinegar samples containing 4.10% and 5.2% acetic acid respectively were produced from MS and PD using *Saccharomyces cerevisiae* strain SR 128 (CS 128) and *Acetobacter aceti* strain WI (AAWI). There was no significant difference (P> 0.05) between body weights, organ weights and organ functions of the experimented rats and the control (normal) rats. Therefore, the prepared vinegar samples from MS and PD had stipulated levels of acetic acid, and they were safe for human consumption, and the sample prepared from PD was slightly better.

Keywords: *Vinegar, Phytochemical, Antimicrobial, Saccharomyces, Acetobacter, Staphylococcus, Escherichia, Candida*

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Introduction

The word Vinegar emanated from the French word *Vin* (wine) and *Aigre* (sour). Vinegar is a sour and sharp liquid used as a condiment and food preservative (Henke *et al.*, 2019). Vinegar can also be viewed as an acetic acid produced through fermentation using an ideal substrate of natural origin, which contains starch or sugar as a carbon source, also fit for human consumption (Bhat *et al.*,2014). Some of the substrates used for vinegar production include rice, grapes, malt, apple, honey, potatoes, whey, and other foods that contain sugar, especially sucrose (Tumane *et al.*, 2018). Acetic acid is the essential flavour and antimicrobial component in vinegar (Matthew *et al.*, 2019). The origin of vinegar can barely be differentiated from the origin of wine (Jia *et al.*, 2012). Although vinegar had been numbered among the lowest quality products of fermented foods, it had also been used as a food condiment, as preservative agent, and in some countries as a healthy juice (Cusano *et al*., 2020).

Vinegars are commonly used for preserving of fruits and vegetables, and in the preparation of salad dressings, mustard, and other food condiments (Jia *et al*., 2012). Although useful as food ingredient for flavour and functional properties, the possible health benefits of vinegar varieties are leading researchers to further consider this long used food product (Cusano *et al*., 2020). Regular consumption of bioactive substances is promoted by many nutritional researchers and the functional food properties of vinegar have been reported in a variety of scientific documentation (Hafzan *et al*., 2017; Purugganan, 2015; Cussano *et al*., 2020).

With the report of the health benefits of vinegar, a concomitant upsurge in demand for fruit vinegar production has ensued (Hazzouri *et al.*, 2015). Medicinal features of vinegar include antibacterial activity, blood pressure reduction, antioxidant activity, reduction in the effects of diabetes, prevention of cardiovascular disease, and increased vigor after exercise (Bhat *et al.*, 2014; Henke *et al.*, 2019; Tumane *et al*., 2018).

Research had shown that vinegar can be produced using biological method (Madrera *et al.*, 2017). Biological production of vinegar involves agricultural-based fruits as source of fermentable sugar and microorganisms such as *Saccharomyces cerevisiae*, acetic acid bacteria such as *Acetobacter aceti* (Matthew *et al.*, 2019). Some of the substrates that are mostly optimized in vinegar production are orange, date, apple, pineapple, mango etc. The sugar molecules in the substrates undergo alcoholic fermentation to produce ethanol (Matthew *et al*., 2019).

The fermentation process is facilitated by the *S. cerevisiae*. The ethanol yielded is acted upon by *Acetobacter aceti* to produce acetic acid, which is known as vinegar (Bhat *et al*., 2014). The significance of the low pH of vinegar is enormous, for instance; it confers preservative property to the product due to inability of spoilage microorganisms to proliferate in acidic environment. Its nutritious and medicinal value had been documented by several researchers (Madrera *et al.*, 2017; Jin *et al*., 2019; Cusano *et al*., 2020).

Research has shown that certain phytochemical compounds in vinegar are capable of harming vital organs in living organisms (Johnston, 2009). According to Johnson (2009), vinegar is widely used as food grade globally, which confers little or no toxicity to it, especially when the substrates are of natural origin. However, heavy intake of vinegar with an acetic acid concentration greater than 20% may affect oral health and the gastrointestinal tract of humans. In a study conducted by Loke *et al*. (2016), tooth erosion was attributed to major consequence of the overconsumption of vinegar. Vinegar is an acidic condiment with a pH value of less than 3.0. Naturally, substances with high acidity contribute to dental erosion or dissolution, thus causing an irreversible loss of teeth enamel and dentin (Gambon *et al.*, 2012).

Researchers have reported that the primary composition of vinegar is acetic acid (Ozturk *et al.*, 2015). The antimicrobial and anti-infective characteristics of vinegar are principally due to the organic acids such as polyphenols and melanoidins. Johnson (2009) also reported that vinegar is capable of exhibiting both bacteriostatic and bacteriocidal activities, especially against food pathogens such as *Escherichia coli*, *Salmonella*, and the causative agent of tuberculosis otherwise known as *Mycobacterium tuberculosis*.

Several researchers have studied the toxicity of vinegar produced from natural substrates such as Johnston (2009), Gambon *et al*. (2012), Ozturk *et al*. (2015), Loke *et al*. (2016), and Anderson *et al.* (2020) but little information had been documented on the safety of vinegar produced from *Phoenix dactylifera* and *Malus sylvestris* in regard to toxicity and acetic acid content. Hence, the aim of this study is to evaluate the safety of vinegar produced from *Phoenix dactylifera* and *Malus sylvestris* in regard to toxicity and acetic acid content.

MATERIALS AND METHODS

Isolation and Characterization of Saccharomyces species from Spoilt Fruit Samples

Sample collection

This was done using a method described by Iheulwumere *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; this was 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 the method described by Iheukwumere *et al*. (2025). The fruit samples were thoroughly washed using distilled water and their ectocarps were appropriately peeled using a stainless chicken knife. The peeled fruits were pulverized using an electric blender (SMX425/Japan). This was serially diluted (1:10) using a 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 thoroughly shaken, stopped and kept for further analysis.

Isolation of yeast

This was done using the 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 into 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 described by 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 on 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), and Iheukwumere *et al*. (2018c). The presence or absence of a 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 a motility test as described by Cheesbrough (2010). The capability of the isolates to produce catalase, indole, oxidase, acetoin, grow in 6.55 % NaCl and utilize sugars, sugar alcohols and other substances (ribose, sorbitol, arabinose, saccharose, 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 sack 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.

Acetic Acid Assay of the Vinegar

This was carried out using the method described in the study published by Onuorah *et al.* (2016). 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:

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

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).

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).

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).

Liver function test

Aspartate aminotransferase (AST) activity: This was carried out as described by Nwobodo *et al*. (2018). 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). 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 37^0C.

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).

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 the 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, 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, 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, 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 7). Tables 8-10 presents the result of organ weight, organ function and metal content of the vinegar sample

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	VA (%)	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)	VA(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)	VA(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
VA	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	VA	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

The presence of *Saccharomyces cerevisiae* strain Ysr128 (SC 128) from the spoilt banana samples corroborated with the findings of Jayamma *et al*. (2020). 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; Petruzziello *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.

The normal and progressive increase in body weights and organ weights in the present study show that the vinegar samples were safe for human consumption. Also, the normal level of liver enzymes, urea, and creatinine conform with the safety of the vinegar samples. The presence of heavy metals that were within the NIS stipulations also conforms to the safety of the vinegar samples. The metal values conform to the NIS standard but disagrees with the values detected by Morhtar *et al*. (2016), and the values were slightly close to the values of vinegar sample VS, commercially produced vinegar bought from the supermarket. The presence of sodium in the vinegar samples increases the health benefit of the vinegar.

CONCLUSION

The study has shown that the prepared vinegar samples from *Malus sylvestris* (MS/Apple) and *Phoenix dactylifera* (PD/Date) fruits contained stipulated acetic acetic level, and they safe for human consumption, and the sample prepared from PD was slightly better.

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Conflict of interests

The authors declare that they have no conflict of interests.

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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.

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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