Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2025, Volume 16, Issue 3, Page 509-521

http://www.jmaterenvironsci.com

Physiochemical Properties of Roselle Calyx (*Hibiscus Sabdariffa*) Wine Produced from Palm Wine and Baker's Yeast (*Saccharomyces cerevisiae)*

Eli Z. W. [1]*, **Alexander D. P.**[2]

[1]*College of Food Science and Technology University of Mkar, Mkar P.M.B 017 Gboko Benue State Nigeria.*
[2]*Centre for Food Technology and Research (CEFTER), Benue State University, Makurdi, Benue State Nigeria*

Corresponding author email: elizughumnaanwell@gmail.com

Received *04 Feb 2025,*
Revised *18 Mar 2025,*
Accepted *18 Mar 2025*

Keywords:
- ✓ *Hibiscus sabdariffa*
- ✓ *Roselle wine;*
- ✓ *Fermentation;*
- ✓ *Physicochemical properties;*
- ✓ *Roselle wine*

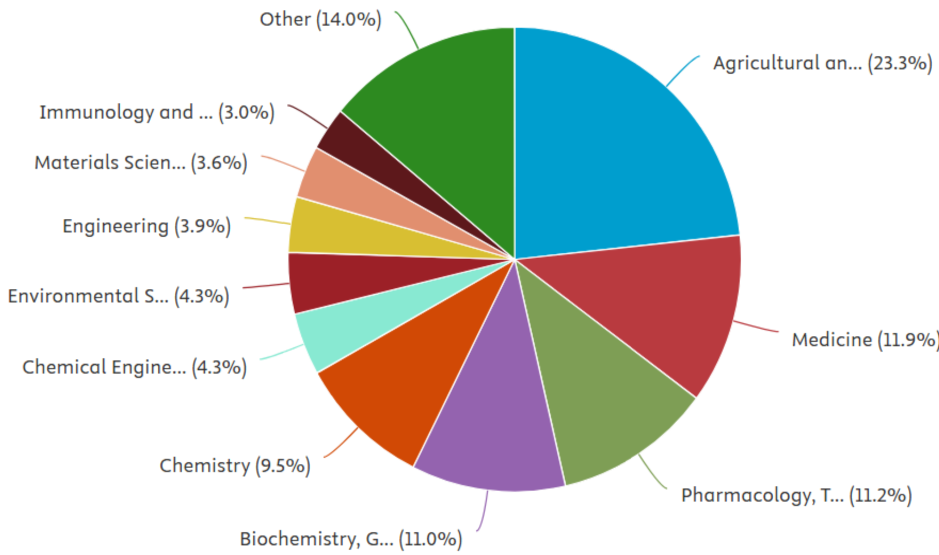
Citation*: Eli Z.W., Alexander D.P. (2025) Physiochemical Properties of Roselle Calyx (Hibiscus Sabdariffa) Wine Produced from Palm Wine and Baker's Yeast (Saccharomyces cerevisiae), J. Mater. Environ. Sci., 16(3), 509-522*

Abstract: This study evaluates the physicochemical properties of roselle (Hibiscus sabdariffa) calyx wine produced through fermentation with palm wine yeast (Saccharomyces cerevisiae) and Baker's yeast. The fermentation process resulted in significant changes in key parameters, with titratable acidity ranging from 0.45% to 0.78%, indicating the potential for long-term storage and a well-defined flavor profile. Vitamin C content varied across samples, with the highest concentration measured at 8.36 mg/100 ml, showcasing the nutritional value of the wine. Total soluble solids (Brix) were monitored, revealing a successful conversion of sugars during fermentation, ultimately contributing to the product's sensory characteristics. Moreover, a notable alcohol concentration of 8% was achieved, aligning with the growing demand for low-alcohol beverages. This study underlines the role of varied fermentation environments in shaping the wine's quality and flavor, presenting roselle calyx wine as a promising functional beverage. The findings support the economic viability of producing roselle wine in regions rich in Hibiscus sabdariffa, enabling local entrepreneurship and export opportunities. Overall, this research contributes valuable insights into the development of innovative beverages and the implications of indigenous food systems.

1. Introduction

The increasing interest in non-alcoholic and low-alcohol beverages has led to the exploration of various natural products and fermentation techniques to produce functional drinks with desirable physicochemical and sensory properties. One such promising source is *Hibiscus sabdariffa* (roselle), known for its rich phytochemical profile, which includes anthocyanins, organic acids, and flavonoids, offering potential health benefits alongside its distinctive tart flavor and vibrant color. Roselle calyxes are widely used in traditional beverages such as "zobo" in Nigeria and have gained global attention as a key ingredient in the development of novel beverages with unique sensory characteristics (Shruthi *et al*., 2016; Izquierdo-Vega *et al*., 2020).

The examination of Scopus indicated that there are 2860 documents on *Hibiscus sabdariffa* subdivided on several fields of interest as Agriculture (23.3%), Medicine (11.9%), Pharmacology (11.2%) etc as shown in **Scheme 1**.



Scheme 1. Repartition of research interest on *Hibiscus sabdariffa*

The fermentation of *Hibiscus sabdariffa* calyxes can significantly enhance the flavor and bioactive content of the beverage, and different fermentative yeasts and substrates can influence the outcome of this process. The use of *Saccharomyces cerevisiae*, a well-known yeast strain, is prevalent in the production of alcoholic and non-alcoholic fermented beverages, where it contributes to both the fermentation dynamics and the final product's organoleptic properties (Çelik *et al*., 2019). Additionally, the role of palm wine, a traditional alcoholic beverage made from the sap of various palm species, has been increasingly explored as a source of fermentative yeast and as a potential substrate for wine production, offering distinct flavors and enhancing the overall fermentation process (Ire *et al*., 2020).

In the current study, we explore the physicochemical properties of roselle calyx wine produced by fermenting Hibiscus sabdariffa calyces using palm wine and Saccharomyces cerevisiae as a fermentation system. We aim to evaluate the impact of this fermentation combination on key parameters, including acidity, sugar content, alcohol concentration, and sensory characteristics, providing new insights into the feasibility of combining roselle with indigenous fermentation practices to develop a functional beverage with enhanced appeal. This manuscript builds on previous work on roselle wine and palm wine fermentation (Sobowale *et al*., 2021; Gutiérrez-Salomón *et al*., 2022), and it aims to further our understanding of how these interactions can lead to the development of an innovative and sustainable product.

Our research also considers the broader implications of this beverage within the context of local agricultural practices and the growing interest in indigenous food systems. The production of roselle wine could support economic growth in regions where Hibiscus sabdariffa is abundant, with potential for local entrepreneurship and export opportunities (Ferrari *et al*., 2022). Furthermore, this study provides a platform for advancing knowledge on the microbial fermentation of roselle calyxes, ultimately contributing to the development of a functional, value-added product in the global beverage market.

2. Methodology

2.1 Sourcing and preparation of materials

Dried Roselle calyx (Dark red cultivar) was purchased at Jos Main Market, while palm wine sample was collected fresh from the tappers early in the morning at Mkar Gboko Local Government Area of Benue State. The palm wine Sample was appropriately labelled with location, date and time of collection. The sample was immediately transported to the laboratory for analysis to obtain the palm wine yeast, (*Saccharomyces cerevisiae)* and the yeast was grown in the Microbiology Laboratory of University of Mkar. Honey was purchased from a bee keeper at Mkar while Dangote sugar and Baker's yeast were purchased from Gboko Main Market and were used

2.1.1 Preparation of the Roselle calyces extracts

Preliminary operations such as cleaning and sorting were carried out to remove extraneous materials from the Roselle calyx. The aqueous extract of Roselle calyx was obtained by boiling 10g of Roselle calyx at a temperature of 100°C for 20 min in a steam jacketed kettle and filtration was done. The dilution factor was 10g of Roselle calyx per 1L of hot water. The juice was filtered from the calyx and 0.33g of sulphur (IV) oxide in the form of sodium metabisulphite was added to the juice to inhibit the growth of bacteria and wild yeast. 200 g, 240g, 200ml, 240ml of sucrose and honey was added respectively to the juice to adjust the soluble solids °Brix. The juice was then poured into an aspirator (fermenting vessel) and the palm wine yeast (*S. cerevisiae*) and baker's yeast was pitched into the juice at room temperature 30C. It was covered for about 15 to 20 min to allow for the yeast population to build up. The fermenting vessel was covered with a safety lock which had 0.1g of sodium metabisulphite at the lid of the lock to control oxidation. The primary fermentation was done for 3 days while secondary fermentation was for 5 days and the ageing of the wine samples was done for 60 days. The experimental design used is completely randomized experimental design.

2.1.2 Palm wine yeasts isolation and identification

Potato Dextrose Agar (P.D.A) was prepared; plates were poured and allowed to solidify. Palm wine samples were streaked on the P.D.A poured plates and was incubated at 28°C four days (Okafor, 1972; Stanly Pradeep *et al*., 2013; Chenaoui *et al*., 2017). Yeast colonies were further sub-cultured on PDA by streaking to obtain pure cultures of palm wine yeast (*Saccharomyces cerevisiae)*. Standard morphological and physiological methods and identification keys described by Phaff and Starmer (1987) were used.

Roselle calyx

↓ Sorting

Clean Roselle Calyx

↓ + Boiled water for 20 minutes

Filtration

↓ Filtrate +Sugar or Honey+ sodium metabisulphite

Fortification/pitching of yeast

↓

Primary Fermentation

↓

Filtration

↓

Secondary Fermentation

↓

Filtration

↓

Clarification/Stabilization, Packaging and Pasteurization

↓

Ageing

Figure 1. Production of Wine Using Roselle Calyx

Samples labeled:

A1: Roselle + Sugar
A2: Roselle + honey
B1: Roselle + Sugar + sodium metabisulphite
B2: Roselle + honey + sodium metabisulphite
C1: Roselle +honey + sugar
C2: Roselle + honey +sugar +sodium metabisulphite
D1: Roselle + baker’s yeast + sugar + sodium metabisulphite

D2: Roselle + palm wine yeast + sugar + sodium metabisulphite
E1: Roselle + baker's yeast + honey + sodium metabisulphite
E2: Roselle + palm wine yeast + honey + sodium metabisulphite
F1: Roselle + baker's yeast + sugar
F2: Roselle + palm wine yeast + sugar
G1: Roselle + baker's yeast + honey
G2: Roselle + palm wine yeast + honey

Palm wine

↓

Preparation of potato dextrose agar

↓

Pouring of plates

↓

Solidification of plates and incubating at 25^0C for 40 minutes

↓

Streaking of the palm wine

↓

Incubating for 4 days

↓

Sub-culturing to obtain pure colonies of palm wine yeast

↓

Colonies of palm wine yeast

Figure 2. Isolation of Palm Wine Yeast

2.2 Determination of Vitamin C

Preparation of reagents

For the determination of vitamin C, a method described by Ranganna, (1986) was used. Starch indicator of 5% was prepared by adding 0.50g soluble starch to 50 ml of distilled water. This was mixed well and allowed to cool before usage. Iodine was prepared by mixing 5.00g of potassium iodide (KI) and 0.268g of potassium iodate (KIO_3) and dissolving 200ml distilled water in a 500ml beaker with. Thereafter 30 ml of 3Molar sulphuric acid was added into the beaker and then diluted with distilled water to the 500ml mask. Vitamin C standard solution was prepared by dissolving 0.250g ascorbic acid with 100ml distilled water in a beaker. The solution was transferred into 250ml container. Standardization of iodine solution with vitamin C standard solution was done by pipetting 25ml of vitamin C solution into a volumetric flask. Five drops of 5% starch solution were added and then titrated against iodine solution until blue black colour was observed. Titrations were done in triplicates. The volume of each sample of wine was measured and the concentration of ascorbic acid per 100ml wine was calculated using the relationship:

$$\text{Concentration of ascorbic acid used in mg/100ml} = \frac{\text{Concentration (mg/ml) standard}}{\text{Weight of samples in grams}} \; x\; 1000$$

2.3 Physicochemical Analyses of the Roselle Wine

Determination of pH

pH determination was done by the method described by Ojokoh & Orekoya (2016). The pH meter was calibrated at 30°C using buffer solutions of acidic and basic values of 4.01 and 8.06 respectively.

Determination of temperature

The temperature was determined using a digital thermometer. The screen of the thermometer was clear before the measurement and the thermometer alert the researcher once the reading is completed (Henderson, 2019).

Determination of total soluble solids (TSS)

The TSS content was determined as total sugar concentration in the wine using an Atago hand refractometer (RX 5000, Atago, Tokyo, Japan). One ml of wine was placed at the screen of a calibrated hand refractometer. The readings were taken and results expressed in ºBx.

Determination of total titratable acidity (TTA)

Total titratable acidity analysis was done using AOAC, 2012 method. Exactly 10 ml of wine sample was pipetted and diluted with 200ml of distilled water and 20ml of the diluent was titrated with 0.1N NaOH, 3 drops of 1% phenolphthalein indicator was used. Titration was done using 0.1N NaOH to a persistent faint pink colour compared against a white background. The titre volume was noted and used for calculations of TTA which was expressed as percentage tartaric acid. The acidity of red wine samples was calculated using the formulae (Lum Eisenman, 1999):

$$\text{TTA (\%)} = 0.15 \times \text{milliliters of NaOH used. OR}$$

$$\text{\% TTA} = \text{Titre value} \times 0.1\text{N (NaOH)} \times \text{M.equiv-Tartaric} \times \frac{100}{volume\ of\ sample}$$

Specific Gravity

This was determined using a density bottle. The samples were poured into a 50ml density bottle and weighed. Each weight is known as the mass. The mass was divided by the volume of the density bottle, to get the density. The specific gravity was calculated as shown below

$$\frac{Density\ of\ sample}{\text{Density of water}} = \frac{x(\frac{g}{ml})}{0.998(\frac{\text{g}}{\text{ml}})}$$

where $x = \frac{W_2 - W_1}{\text{Vml}}$, and W_2= weight sample in the density bottle, while W_1= weight of density bottle and V= volume of the density bottle (50ml).

Statistical analysis of Data

All the data were analyzed using the SPSS statistical software. Data means is compared using one-way analysis of variance (ANOVA). The data is analysed for variance using the Duncan multiple range test (Duncan, 1955) to locate differences among sample means at 5% level of significance, least significant difference (LSD) was used to determine significant results. Difference between the samples were considered statistically significant at P<0.05.

3. Results and Discussions

3.1 Results

Vitamin C Content of the Wine is given at Table 1 and changes in Specific Gravity of the Wine is presented at Figure 3.

Table 1: Vitamin C content of the wine (mg/100ml)

Sample	Vitamin C
A1	$8.03^{a} \pm 0.057$
A2	$8.10^{a} \pm 0.100$
B1	$7.93^{a} \pm 0.057$
B2	$6.60^{f} \pm 0.000$
C1	$6.73^{e} \pm 0.057$
C2	$8.00^{a} \pm 0.100$
D1	$7.90^{a} \pm 0.100$
D2	$8.36^{a} \pm 0.577$
E1	$7.20^{e} \pm 0.000$
E2	$7.60^{b} \pm 0.100$
F1	$6.41^{g} \pm 0.115$
F2	$7.83^{a} \pm 0.057$
G1	$8.00^{a} \pm 0.000$
G2	$6.96^{d} \pm 0.057$

(Mean ± standard deviation triplicates)

*a, b, c, d, e means with different superscripts are significantly different (p<0.05) but *a, b, c, d, e means with the same superscripts are not significantly different.

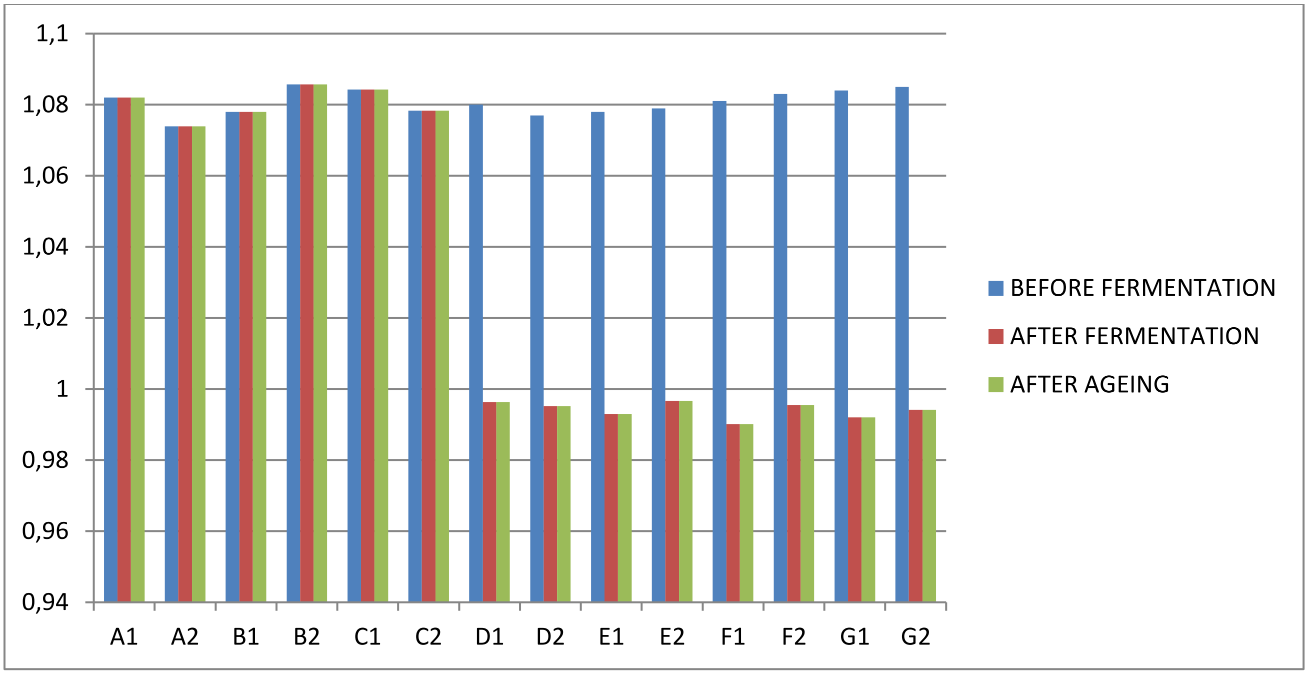


Figure: 3 Graphical representations of the changes in specific gravity of the wine before fermentation, after fermentation and after ageing

Changes in Total Soluble Solids (Brix)

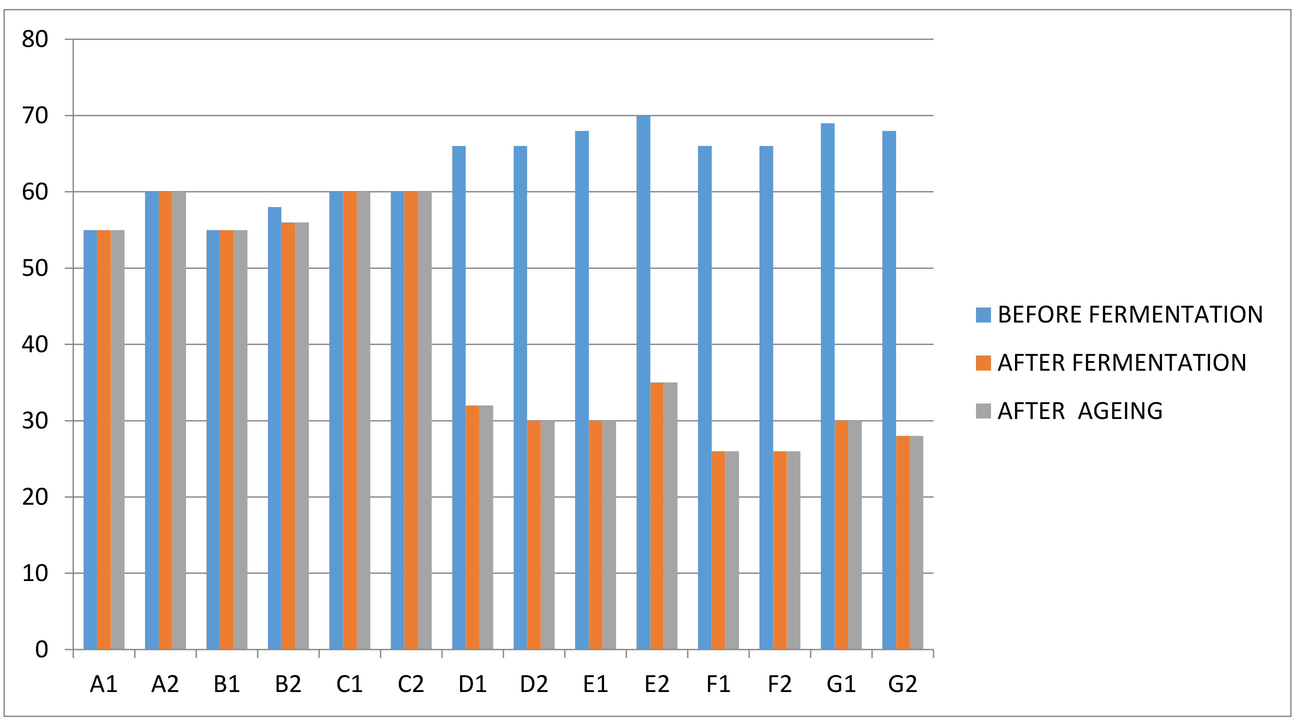


Figure: 4 Graphical representations of the changes in total soluble solids (brix) of the wine before fermentation, after fermentation and after ageing

Changes in pH of the Wine

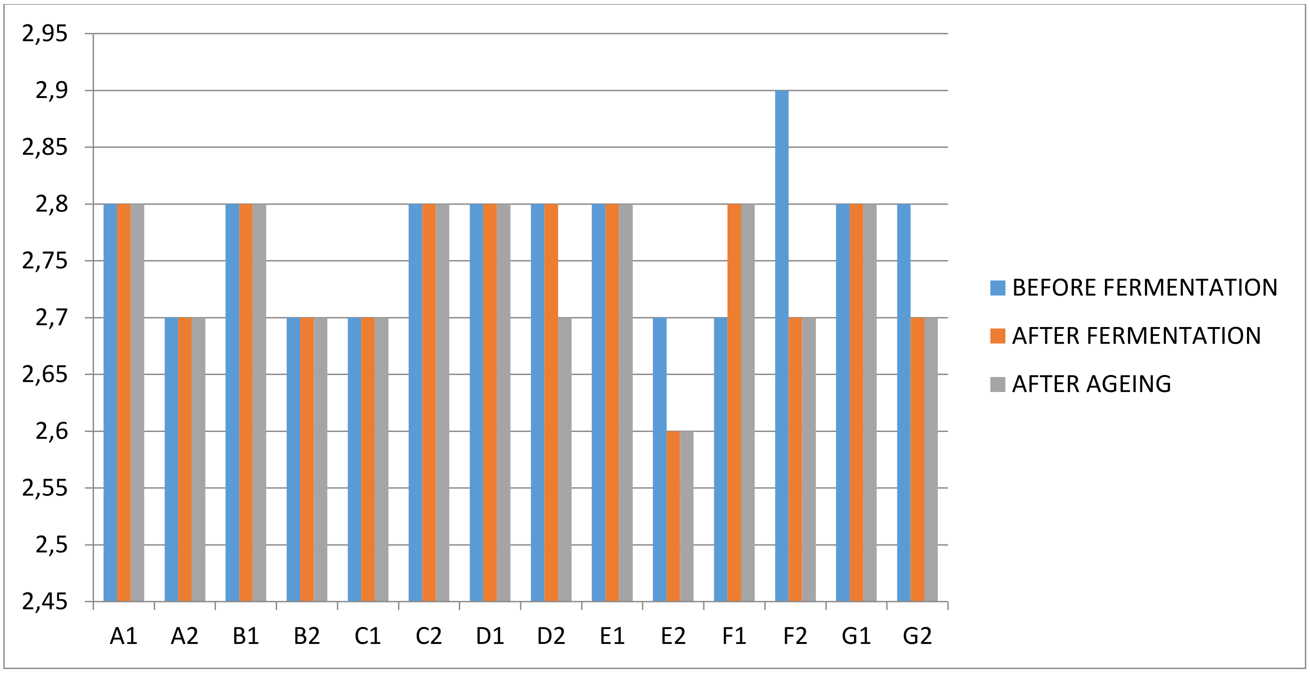


Figure: 5 Graphical representations of the changes in pH of the wine before fermentation, after fermentation and after ageing

Changes in Temperature of the Wine

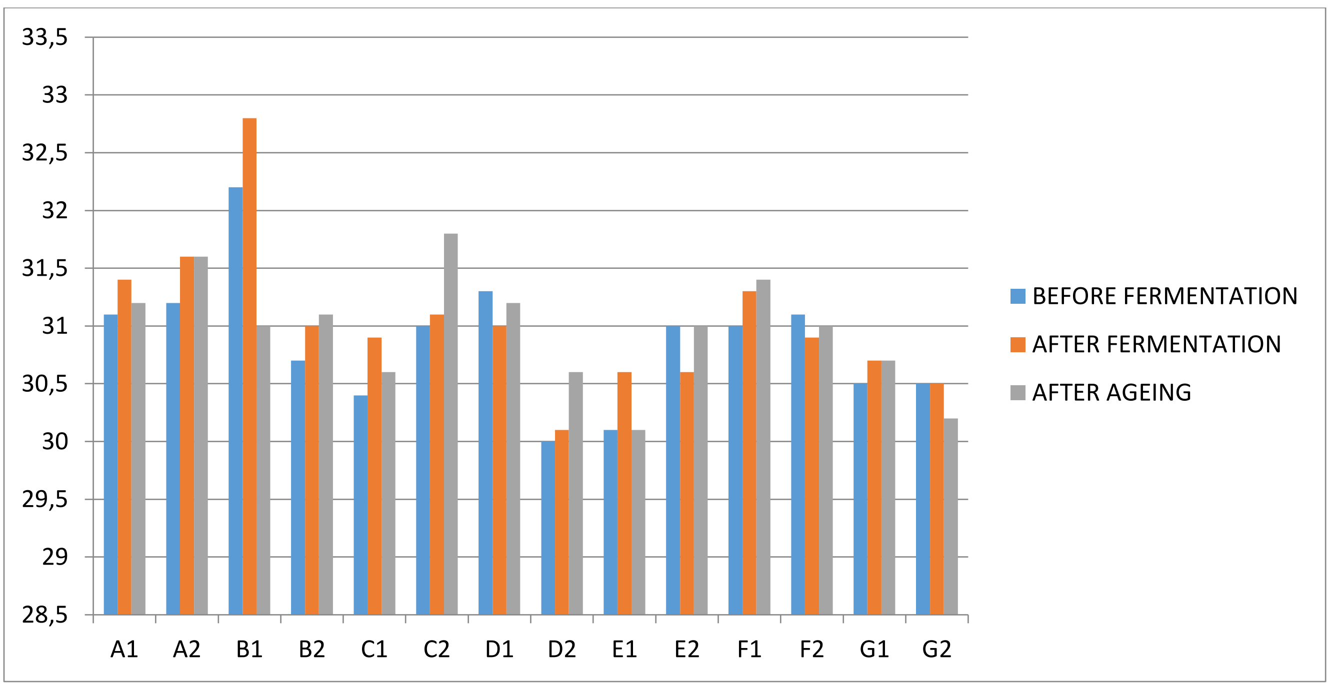


Figure 6: Graphical representations of the changes in temperature of the wine before fermentation, after fermentation and after ageing (°C)

The Total Titratible Acidity in the Wine

Table 2: Total titratible acidity

Sample	% Titratable acids
A1	$0.71^{c} \pm 0.0058$
A2	$0.67^{g} \pm 0.0058$
B1	$0.68^{f} \pm 0.0058$
B2	$0.69^{f} \pm 0.0058$
C1	$0.67^{g} \pm 0.0058$
C2	$0.67^{g} \pm 0.0058$
D1	$0.70^{d} \pm 0.0057$
D2	$0.70^{cd} \pm 0.1000$
E1	$0.69^{ef} \pm 0.0057$
E2	$0.70^{cd} \pm 0.000$
F1	$0.72^{c} \pm 0.0057$
F2	$0.74^{a} \pm 0.0057$
G1	$0.74^{a} \pm 0.0057$
G2	$0.75^{a} \pm 0.0057$

(Mean ± standard deviation triplicates)
*a, b, c, d, e means with different superscripts are significantly different ($p<0.05$) but *a, b, c, d, e means with the same superscripts are not significantly different.

3.2 Discussions

The present study provides a comprehensive analysis of the physicochemical properties of roselle (*Hibiscus sabdariffa*) calyx wine produced using palm wine and *Saccharomyces cerevisiae*. Several key parameters were measured, including vitamin C content, specific gravity, total soluble solids (Brix), pH, temperature, and titratable acidity, to assess the effects of fermentation and ageing on the quality and stability of the wine. These parameters are important for understanding the nutritional value, sensory properties, and overall quality of the wine.

Vitamin C Content

The results show that the vitamin C content of the wine ranged from 6.41 mg/100ml to 8.36 mg/100ml (Table 1). Notably, samples A1, A2, B1, C2, D1, D2, F2, and G1 exhibited the highest vitamin C levels, while samples B2, C1, E1, E2, and F1 were significantly lower in vitamin C content. The retention of vitamin C in the wine after fermentation is crucial since this vitamin is known for its antioxidant properties, which can help improve health by neutralizing free radicals in the body (Apaliya *et al*., 2021). The variations in vitamin C content between samples may be attributed to differences in fermentation conditions, such as yeast strain, fermentation time, and temperature, which are known to influence the stability of bioactive compounds (Sobowwale *et al*., 2021).

Changes in Specific Gravity

The specific gravity of the wine samples was observed to decrease after fermentation and ageing (Figure 3). This decrease is characteristic of the fermentation process, where sugars are converted into alcohol by the yeast (*Saccharomyces cerevisiae*), leading to a reduction in the wine's density. The absence of specific gravity changes in non-fermented samples further supports the role of yeast in reducing the specific gravity through fermentation. The changes in specific gravity align with findings from previous studies on other fruit-based wines, where a similar reduction in specific gravity occurs due to sugar consumption during fermentation (Ezemba *et al*., 2022).

Changes in Total Soluble Solids (Brix)

The total soluble solids (Brix) of the wine samples decreased in most of the samples after fermentation (Figure 4). Brix values are indicative of the sugar concentration in the wine, and the observed decrease in Brix is consistent with the fermentation process, where sugars are converted into alcohol (Purkait & Pandey, 2020). However, some samples, such as B1, B2, and C2, did not show a significant decrease in Brix values. This could be due to variations in the fermentability of the sugars in the roselle calyx and palm wine mixture or differences in the yeast's ability to metabolize these sugars (Lu *et al*., 2017). It is noteworthy that the decrease in Brix is an important indicator of successful fermentation, influencing the sweetness and alcohol content of the final wine.

pH Changes

The pH of the wine samples ranged from 2.6 to 2.8 throughout the fermentation and ageing processes (Figure 5). The relatively low pH of the wine suggests the presence of organic acids, such as citric and tartaric acids, which contribute to the overall acidity of roselle-based beverages. This acidity is essential for preserving the wine and contributes to its tart and refreshing flavor profile (Onuoha *et al*., 2014). The stability of pH across different phases of fermentation and ageing indicates that the wine’s acidity remains relatively unaffected by the fermentation process, which is a positive characteristic for the wine’s long-term preservation.

Temperature Changes

The temperature data presented in Figure 6 indicates that samples A1, A2, B1, B2, C1, and C2 experienced an initial increase in temperature due to the use of hot water during the extraction process. However, the temperature of these samples subsequently decreased to room temperature, aligning with the cooling process after fermentation. The temperature changes observed during fermentation are typical of microbial activity, where heat is generated as the yeast ferments the sugars (Drappier *et al*., 2019). The temperature fluctuations also support the idea that proper control of fermentation temperature is crucial for the quality of the wine, as fermentation at higher or uncontrolled temperatures could lead to off-flavors or spoilage (Ibitoye *et al*., 2017).

Titratable Acidity

The total titratable acidity (TTA) of the wine ranged from 0.67% to 0.75% (Table 2), with samples F2, G1, and G2 exhibiting the highest acidity levels. Titratable acidity is a key factor in determining the taste, stability, and preservation of wine. A moderate acidity level is desirable for wine quality as it helps balance sweetness and provides a refreshing and crisp taste (Sobowale *et al*., 2021). The relatively high levels of titratable acidity in some samples suggest that the wine has good potential for long-term storage and could have a well-defined flavor profile. The differences in acidity between samples may be influenced by variations in the fermentation environment, yeast strain, and the composition of the starting materials, such as palm wine and roselle calyces (Ezemba *et al*., 2022; Ogonna *et al*., 2018).

The physicochemical results from this study demonstrate that roselle calyx wine produced through fermentation with *Saccharomyces cerevisiae* and palm wine exhibits a range of beneficial properties, including retention of vitamin C, moderate acidity, and successful sugar conversion during fermentation. These factors contribute to the wine's nutritional value, sensory appeal, and potential for long-term preservation. The findings align with previous research on roselle-based beverages and fermented wines, highlighting the importance of fermentation conditions in determining the final wine quality (Purkait & Pandey, 2020; Onuoha *et al*., 2014). The observed variations in some parameters, such as vitamin C content and acidity, suggest that optimizing fermentation conditions and yeast strain selection could further enhance the quality and consistency of roselle wine. This study opens the door for further exploration into the use of roselle as a base for functional and nutritious fermented beverages, potentially contributing to sustainable, health-promoting wine production.

Conclusion

The study demonstrates that roselle (Hibiscus sabdariffa) calyx wine, produced through fermentation with palm wine and Saccharomyces cerevisiae, exhibits favorable physicochemical properties that enhance its sensory appeal and nutritional value. Key findings highlight the wine's moderate acidity, retention of vitamin C, and successful sugar conversion, which collectively contribute to its potential as a functional and health-promoting beverage. The variations in wine quality based on fermentation conditions suggest opportunities for optimization, indicating that further exploration in this area could lead to improved consistency and product development. Ultimately, the findings position roselle wine as a viable option for sustainable beverage production, encouraging local entrepreneurship while enriching the beverage market with innovative and nutritious offerings.

Acknowledgement

The technical inputs of Mr Bem Gbusu of the College of Food Science and Technology University of Mkar, Benue State Nigeria for his Technical support.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References

Ani, C. P. (2021). Production, sensory and physicochemical evaluation of zobo and zobo-date wine from Hibiscus sabdariffa flower, pineapple, orange and lime juice using Saccharomyces cerevisae. *International Journal of Applied Chemical and Biological Sciences*, 2(4), 100-110.

OAC. (2012). Association of Official Analytical Chemist. Official methods of analysis of the Analytical Chemist International, 18th ed. Gathersburg, MD USA.

AOAC. (2012). Association of Official Analytical Chemist. Official Methods of Analysis of the Analytical Chemist International, 18Thed. Gathersburg, MD USA.

Apaliya, M. T., Kwaw, E., Mahunu, G. K., Osei-Kwarteng, M., Osae, R., & Azirigo, M. (2021). Nutritional properties and feeding values of Hibiscus sabdariffa and their products. In *Roselle (Hibiscus sabdariffa)* (pp. 137-154). Academic Press. https://doi.org/10.1016/B978-0-12-822100-6.00006-9.

Çelik, Z. D., Erten, H., & Cabaroglu, T. (2019). The influence of selected autochthonous Saccharomyces cerevisiae strains on the physicochemical and sensory properties of narince wines. *Fermentation*, 5(3), 70. https://doi.org/10.3390/fermentation5030070.

Chenaoui M., Amar M., Benkhemmar O., El Aissami A., Arahou M., Rhazi L. (2017), Isolation and characterization of fungi from sugar beet roots samples collected from Morocco, J. Mater. Environ. Sci. 8 (11), 3962-3967.

Drappier, J., Thibon, C., Rabot, A., & Geny-Denis, L. (2019). Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming. *Critical Reviews in Food Science and Nutrition*, 59(1), 14-30. https://doi.org/10.1080/10408398.2017.1355776 .

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1-42. https://doi.org/10.2307/3001478.

Eisenman, L. (1999). The home winemakers manual (pp. 151-152).

Etiévant, P. X. (2017). Wine. In *Volatile Compounds in Foods and Beverages* (pp. 483-546). Routledge. https://doi.org/10.1201/9780203734285-14.

Ezemba, C. C., Anakwenze, V. N., & Ezemba, A. S. (2022). Wine production from apple (Malus pumila) using yeast isolated from palmwine. *Current Journal of Applied Science and Technology, 41*(3), 16. https://doi.org/10.9734/cjast/2022/v41i331653.

Ferrari, S., Nicotera, T., lo Presti, A., & Biasone, A. M. (2022, May). Local development through the connection between roots tourism, local food and wine. In *International Symposium: New Metropolitan Perspectives* (pp. 2301-2312). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-06825-6_221.

Gutiérrez-Salomón, A. L., Barajas-Ramírez, J. A., Aguilar-Raymundo, V. G., & Castaneda Ovando, A. (2022). Influence of keeping the calyces during fermentation on physicochemical and sensory properties of Hibiscus sabdariffa wines. *Journal of Food Science and Technology*, 1-11. https://doi.org/10.1007/s13197-021-05056-x.

Henderson, K. O. (2019). Temperature measurement. In Fuels and Lubricants Handbook (p. 1269). ASTM International.https://doi.org/10.1520/MNL3720160031.

Ibitoye, F., Oyetayo, A., Aribisala, O., & Giwa, O. (2017). Investigation of bacteria associated with the spoilage of zobo drink fortified with scent leaf and ginger. *Archives of Current Research International*, *9*(1), 1-7. https://doi.org/10.9734/ACRI/2017/35415.

Ire, F. S., Okeke, J. I., & Maduka, N. (2020). Suitability and fermentative performance of indigenous palm wine yeast (*Saccharomyces cerevisiae*) using apple. *South Asian Journal of Biological Research*,*3*(2), 55-74.

Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., Vargas Mendoza, N., Gómez-Aldapa, C. A., ... & Madrigal-Santillán, E. (2020). Organic acids from Roselle (Hibiscus sabdariffa L.)-A brief review of its pharmacological effects. *Biomedicines*, *8*(5), 100. https://doi.org/10.3390/biomedicines8050100.

Kantiyok, B. D., Mohammed, S. S. D., & Yabaya, A. (2021). Fruit wine produced from pawpaw (Carica papaya) and watermelon (Citrullus vulgaris) using Saccharomyces cerevisiae isolated from selected fermented foods. *Science World Journal*, *16*(4), 419-427.

Lu, Y., Voon, M. K. W., Huang, D., Lee, P. R., & Liu, S. Q. (2017). Combined effects of fermentation temperature and pH on kinetic changes of chemical constituents of durian wine fermented with Saccharomyces cerevisiae. *Applied Microbiology and Biotechnology, 101*, 3005-3014. https://doi.org/10.1007/s00253-016-8043-1.

Ogonna, U. S., Chukwuma, O. U., Sunday, A. N., Jane, O. C., & Blessing, A. (2018). Preliminary investigation into the use of roselle (*Hibiscus sabdariffa*) and ugiri (*Irvingia gabonesis*) fruits in wine production. *International Journal of Life Science and Engineering, 3*(3), 64-68.

Ojokoh, A. O., & Orekoya, E. S. (2016). Effect of fermentation on the proximate composition of the epicarp of watermelon (Citrullus lanatus). *International Journal of Swarm Intelligence and Evolutionary Computation*, *5*(3), 1-5.

Okafor, N. (1972). Palm-wine yeasts from parts of Nigeria. *Journal of the Science of Food and Agriculture, 23*(12), 1399-1407. https://doi.org/10.1002/jsfa.2740231203.

Onuoha, O. G., Haruna, U. S., Yelmi, B. M., Samuel, E., Uhiara, N. S., & Ngwu, P. C. (2014). Storage study on color retention in zobo concentrates by increasing concentration of ginger (Zingiber officinale). *African Journal of Food Science, 8*, 292-296. https://doi.org/10.5897/AJFS2013.1109.

Phaff, H. J., & Starmer, W. T. (1987). Yeasts associated with plants, insects, and animals. In A. H. Rose& J. S. Harrison (Eds.), *The yeasts: A taxonomic study* (pp. 181-202). Elsevier.

Purkait, T., & Pandey, S. (2020). Development, physicochemical characterization and sensory evaluation of a red wine using roselle (Hibiscus sabdariffa) and peppermint (Mentha piperita L.) extract. *Medico-Legal Update*, *20*(4), 1410-1417.

Ranganna, S. (1986). Handbook of analysis and quality control for fruit and vegetable products (2nd ed.). *Tata McGraw-Hill Education*.

Shruthi, V. H., Ramachandra, C. T., Nidoni, U., Hiregoudar, S., Naik, N., & Kurubar, A. R. (2016). Roselle (Hibiscus sabdariffa L.) as a source of natural colour: *A review. Plant Archives,* 16(2), 515-522.

Sobowale, S. S., Omosebi, O. M., & Animashaun, O. H. (2021). Characterization of Roselle (Hibiscus sabdariffa) calyces wine using date palm (Phoenix dactylifera) fruit extracts as a substitute for granulated sugar. *Journal of Food Processing and Preservation, 45*(11), e15935. https://doi.org/10.1111/jfpp.15935.

Stanly Pradeep F., Shakila Begam M., Palaniswamy M., Pradeep B.V. (2013) Influence of Culture Media on Growth and Pigment Production by Fusarium moniliforme KUMBF1201 Isolated from Paddy Field Soil, *World Applied Sciences Journal* 22(1), 70-77, https://doi.org/10.5829/idosi.wasj.2013.22.01.7265

Tahir, H. E., Mahunu, G. K., Xiaobo, Z., & Mariod, A. A. (2021). Ethnobotanical uses, fermentation studies and indigenous preferences of Hibiscus sabdariffa. In *Roselle (Hibiscus sabdariffa)* (pp. 231-254). *Academic Press*. https://doi.org/10.1016/B978-0-12-822100-6.00013-6.

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2024). Understanding wine chemistry. *John Wiley & Sons*. https://doi.org/10.1002/9781394258406.