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Quantitative microbiological risk assessment, physicochemical quality and sensory profiles of artisanal cashew wine and zobo compared with commercial grape wines in Nigeria

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ABSTRACT

Artisanal fermented and non-alcoholic beverages are widely consumed in Nigeria, yet their microbiological safety is rarely quantified alongside their physicochemical and sensory quality. This study compared artisanal cashew wine and zobo with commercial red and rosé wines. Eight microbial groups were enumerated by spread plating, and the staphylococcal isolate was confirmed as coagulase positive. A growth-coupled quantitative microbial risk assessment used a square-root model with temperature and pH terms and 50,000 Monte Carlo iterations. Physicochemical properties were compared by one-way analysis of variance and principal component analysis, while 20 panelists scored sensory attributes on a 5-point scale. Detected counts ranged from 1.30 to 3.00 log CFU mL^{-1}, and faecal coliforms, moulds and pseudomonads were not detected. Artisanal beverages carried the highest microbial loads, notably coagulase-positive staphylococci in cashew wine (3.00 log CFU mL^{-1}) and acetic acid bacteria in zobo (2.58 log CFU mL^{-1}). At the measured pH of 3.28, no simulated cashew wine serving reached the 5-log toxigenic threshold under any storage scenario, whereas a pH-permissive-worst case at 24 to 34 °C reached it in 72.4% of servings within 12 h. Beverage pH ranged from 3.17 to 3.38, and the first principal component (68.0%) separated sugar-rich zobo from the wines. Zobo scored at least as high as red wine on 12 of 13 attributes. Acidity, rather than contamination level, governed staphylococcal risk, although the staphylococcal count signals post-fermentation hygiene failure.

Keywords: *Staphylococcus aureus; quantitative microbial risk assessment; predictive microbiology; hurdle concept; Hibiscus sabdariffa; cashew apple wine.*

1. Introduction

Traditional and artisanal beverages occupy an important place in Nigerian diets, and many are produced in homes or small enterprises with little regulatory oversight. In a review of nine such beverages, Nwaiwu et al. [1] recorded *Bacillus*, *Escherichia*, *Staphylococcus* and several fungal genera across products, and they observed that hygiene indicators were more prevalent in non-alcoholic drinks than in alcoholic ones. Fermented palm wine follows an ecological course in which *Saccharomyces cerevisiae* dominates while lactic acid bacteria (LAB) and acetic acid bacteria (AAB) shape acidity and flavour [2]. Consequently, the balance between desirable fermentative microbiota, spoilage organisms and pathogens can be expected to differ between artisanal and industrial products.

Zobo, a sweetened infusion of *Hibiscus sabdariffa* calyces, illustrates the problem. Okoi et al. [3] isolated eight bacterial species from zobo hawked in Eket, with *Staphylococcus aureus* the most frequent, and reported bacterial counts in the order of 10^3 CFU mL^{-1}. After six weeks of storage, counts in mulled zobo reached 4.9×10^5 to 7.2×10^5 CFU depending on the container, although the product remained strongly acidic at pH 2.06 to 2.79 [4]. Adisa et al. [5] likewise measured a pH of 2.8 in calyx extracts. The acidity of zobo derives from citric, hydroxycitric, hibiscus, malic and tartaric acids [6], and antimicrobial activity has been reported for calyx extracts of both red and white varieties [7]. Furthermore, LAB isolated from zobo can inhibit *S. aureus* and *Escherichia coli* in vitro [8], which suggests that native microbiota may contribute to product stability.

Cashew apple wine is a second artisanal product of growing interest because the cashew apple, which is discarded after nut harvest, is rich in sugars, ascorbic acid and tannins [9]. According to Akyereko et al. [10], cashew apple juice had a pH of 3.72 and 10.60 °Brix, and earlier studies cited by these authors reported pH values of 4.20 to 4.40. Recent work has also shown that non-*Saccharomyces* yeasts can improve the phenolic bioaccessibility and aroma of cashew wine [11].

By contrast, commercial grape wines sold in Nigeria are presumed to be microbiologically stable, yet Ofoedu et al. [12] found that domestic red wines differed markedly from imported ones in pH (4.03 to 4.16), sugar and alcohol content, which indicates that "commercial" does not guarantee uniform composition.

Among the relevant hazards, *S. aureus* is of particular concern because staphylococcal food poisoning is an intoxication caused by preformed enterotoxins rather than by infection. These enterotoxins resist heat and proteolysis and tolerate a wide pH range [13], and they affect consumers even in nanogram amounts [14]. A meta-analysis found enterotoxins in 27.11% of mixed and ready-to-eat foods contaminated by staphylococci [15], and Okoye and Eruteya [16] detected enterotoxin A and B genes in *S. aureus* from ready-to-eat foods in Rivers State. Enterotoxins are conventionally considered detectable once populations exceed 10^5 CFU g^{-1} [17], whereas recent experiments place the threshold nearer 6 log CFU mL^{-1} [18] or 6.5 to 8.0 log CFU g^{-1} [19]. The risk posed by a given count therefore depends on whether the organism can grow in the product before consumption.

Quantitative microbial risk assessment (QMRA) provides a structured framework, comprising hazard identification, exposure assessment, hazard characterisation and risk characterisation, for converting such counts into risk estimates [20]. For toxin-forming organisms, coupling exposure to a predictive growth model is essential, and the model must respect the organism's growth limits. Jia et al. [21] reported theoretical minimum growth temperatures of 5.0 to 7.2 °C for *S. aureus*, and Haddad et al. [22] noted survival down to pH 4.2 with toxin production restricted to pH 5.3 to 7.0. Low pH and ethanol act as complementary hurdles: pathogens grew in non-alcoholic beer but not in a low-alcohol version [23], and pH was the critical factor for pathogen control in a non-alcoholic beer matrix [24].

Despite these advances, the Nigerian studies cited above report counts without translating them into risk estimates constrained by product pH, temperature and holding time, and without relating safety to physicochemical and sensory quality. This study therefore aimed to (i) enumerate eight microbial groups in two artisanal beverages (cashew wine and zobo) and two commercial grape wines (red and rosé); (ii) assess the staphylococcal risk of cashew wine using a growth-coupled Monte Carlo QMRA that accounts for temperature and pH; (iii) characterise the physicochemical properties of the four beverages using univariate and multivariate statistics; and (iv) compare their sensory profiles.

2. Materials and methods

2.1. Samples and study design

Four beverages were examined [two artisanal products, namely cashew apple wine and zobo (a non-alcoholic *H. sabdariffa* calyx infusion)], obtained from local producers, and two commercial grape wines (red and rosé) purchased from retail outlets in Port Harcourt metropolis. Three sealed bottles of each beverage were collected as independent sampling units, kept in their original containers and transported to the Department of Microbiology's food microbiology laboratory in ice packed cooler. Each bottle was analysed in duplicate.

2.2. Enumeration of microbial groups

Test portions were prepared in accordance with the general requirements of ISO 7218:2024 [25]. Bottle surfaces were disinfected with 70% (v/v) ethanol, the contents were mixed by inversion and opened aseptically, and serial decimal dilutions were prepared in sterile diluent. Aliquots (0.1 mL) of the undiluted sample and of appropriate dilutions were spread in duplicate on the following media: nutrient agar for total heterotrophic bacteria count (THBC) at 37 °C for 24 to 48 h; MacConkey agar at 44 °C for 24 h for faecal (thermotolerant) coliforms count; Sabouraud dextrose agar at 25 °C for 3 to 5 d for yeasts and moulds count; de Man, Rogosa and Sharpe (MRS) agar at 30 °C for 48 to 72 h under microaerophilic conditions in candle jar cannister for LAB count; glucose, yeast extract and calcium carbonate (GYC) agar at 30 °C for 48 to 72 h for AAB count, whose colonies were recognised by clear zones of carbonate dissolution; cetrimide agar at 35 °C for 24 to 48 h for pseudomonad count; and mannitol salt agar (MSA) at 37 °C for 24 to 48 h for staphylococcal count.

Colony counts were converted to colony-forming units per millilitre as

$$N = \frac{\Sigma C}{V \times n \times d} \quad (1)$$

where ΣC is the sum of colonies on the number of plates (n) retained at dilution (d) and V is the inoculated volume (0.1 mL). Results were expressed as $\log_{10}$CFU mL^{-1}. The limit of detection was 10 CFU mL^{-1} (1.00 $\log_{10}$CFUmL^{-1}), corresponding to one colony on a 0.1 mL plate of the undiluted sample, and results below it was recorded as not detected (ND). Counts derived from plates below the recommended countable range were flagged as estimated counts, as required by ISO 7218:2024 [25].

Mannitol-fermenting (yellow) colonies on MSA were counted as presumptive staphylococci, and representative colonies were confirmed by Gram staining (Gram-positive cocci in clusters), catalase production and the coagulase test. Because MSA is not the reference medium specified in ISO 6888-1:2021 [26], which prescribes Baird-Parker agar, the staphylococcal count is reported as a count of presumptive staphylococci whose representative isolate was confirmed as coagulase-positive.

2.3. Quantitative microbial risk assessment

The assessment followed the four-step structure recommended by FAO and WHO [20]. Hazard identification screened each enumerated group for public health relevance. Exposure was estimated as the ingested viable dose, D = C × V (2)

where C is the staphylococcal concentration (CFU mL^{-1}) and V is the serving volume (100, 150, 200 or 250 mL). Staphylococcal food poisoning is toxin-mediated, therefore, hazard characterisation used a population threshold rather than an infection dose–response function: 5.0 $\log_{10}$CFUmL^{-1} was adopted as the primary toxigenic threshold [17], and 6.0 $\log_{10}$CFUmL^{-1} was applied as a sensitivity case [18]. The acceptability of the measured count was also classified against the coagulase-positive staphylococcal criteria of FSANZ [27].

Risk characterisation coupled exposure to a predictive growth model. The maximum specific growth rate (μ_{max}, h^{-1}) was described by a square-root secondary model with temperature and pH terms:

Maximum Specific Growth Rate

For conditions where $T > T_{\min}$ and $\mathrm{pH} > \mathrm{pH}_{\min}$, the growth rate is defined as:

$$\sqrt{\mu_{\max}} = b(T - T_{\min})\sqrt{1 - 10^{(\mathrm{pH}_{\min} - \mathrm{pH})}} \quad (3)$$

For $T \leq T_{\min}$ or $\mathrm{pH} \leq \mathrm{pH}_{\min}$:

$$\mu_{\max} = 0 \quad (4)$$

Lag Time
The lag phase duration is modelled as a function of the physiological work parameter (h_0):

$$\lambda = \frac{h_0}{\mu_{\max}} \quad (5)$$

Three-Phase Linear Growth Model
Population growth over time (t) is modeled as a piecewise function:

$$\log N(t) = \begin{cases} \log N_0, & t \leq \lambda \\ \min\left[\log N_0 + \frac{\mu_{\max}(t-\lambda)}{\ln 10}, \log N_{\max}\right], & t > \lambda \end{cases} \quad (6)$$

Time to Reach Toxigenic Threshold (TTT)
The time required for the population to reach the predefined toxigenic threshold (N_{thr}) is calculated as:

$$\mathrm{TTT} = \lambda + \frac{\ln(10)\,(\log N_{\mathrm{thr}} - \log N_0)}{\mu_{\max}} \quad (7)$$

Anchoring Coefficient (b) Reference
The baseline maximum specific growth rate, utilized to anchor the coefficient b, is established at reference conditions of $T = 37\,^{\circ}\mathrm{C}$ and $\mathrm{pH} = 7.0$:

$$\mu_{\max,\mathrm{baseline}} = \frac{\ln 2}{0.5}\ \mathrm{h}^{-1} \quad (8)$$

Table 1: Input parameters of the growth-coupled Monte Carlo model for coagulase-positive staphylococci in cashew wine

Parameter	Symbol	Value or distribution	Basis
Initial concentration	$\log N_0$	Normal (3.00, 0.10) $\log_{10}$ CFU mL^{-1}	Measured count (990 CFU mL^{-1}); plating uncertainty assumed
Product pH, measured	pH	Normal (3.28, 0.04)	Measured mean ± SD (Section 3.4)
Storage temperature, ambient	T	Uniform (24, 34) °C	Screening scenario
Storage temperature, room	T	Uniform (18, 26) °C	Screening scenario
Storage temperature, cool	T	Uniform (4, 10) °C	Screening scenario
Minimum growth temperature	$T_{\min}$	Uniform (5.0, 7.2) °C	Jia et al. [21]; Bai et al. [28]
Minimum growth pH	$\mathrm{pH}_{\min}$	Uniform (4.0, 4.5)	Brackets the pH 4.2 limit reported by Haddad et al. [22]
Reference growth rate at 37 °C, pH 7.0	μ_{ref}	1.386 h^{-1} × Lognormal (0, 0.15)	Screening scenario; variability assumed
Work to be done during lag	h_0	Uniform (1.5, 4.0)	Assumed; h_0 = 2.77 gives a 2 h lag at 37 °C
Maximum population density	$\log N_{\max}$	9.0 $\log_{10}$ CFU mL^{-1}	Screening scenario
Toxigenic threshold	$\log N_{\mathrm{thr}}$	5.0 (primary); 6.0 (sensitivity) $\log_{10}$ CFU mL^{-1}	Silva et al. [17]; Lee et al. [18]
Iterations per scenario	n	50,000	Random seed 20260928

Note. *SD = standard deviation. The measured pH was used in the baseline simulation; a pH-permissive worst case (pH 7.0) and a pH scan from 4.0 to 7.0 in steps of 0.1 were run as scenario analyses. The probability that a serving reached the threshold was estimated at holding times of 2 to 48 h under the three temperature scenarios. Global sensitivity was assessed with Spearman rank correlation coefficients between each sampled input and the TTT.*

2.4. Physicochemical analyses
Alcoholic strength (% v/v), total nitrogen (Kjeldahl method), pH (calibrated digital pH meter), specific gravity, soluble solids (°Brix, refractometry), total solids (oven drying to constant weight), total titratable acidity (TTA, titration with standard sodium hydroxide) and viscosity were determined in duplicate by standard procedures [29,30]. Because the refractive index of wine responds to sugars, organic acids, glycerol and ethanol [31], the °Brix values of the wines are interpreted as apparent soluble solids.

2.5. Sensory evaluation
Twenty untrained adult panelists evaluated the beverages on a five-point intensity scale in two paired sessions: cashew wine with rosé wine (12 attributes) and zobo with red wine (13 attributes, including acidity). Attributes were grouped into five categories, namely visual, olfactory, mouthfeel, gustatory and overall impression, and category scores were computed as the arithmetic means of their attribute means.

2.6. Statistical analysis
Physicochemical data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test at $\alpha = 0.05$ [statistical package to be inserted]. For variables with a standard deviation for every beverage, F statistics and eta-squared (η^2) were reconstructed from the summary statistics. Pearson correlations, principal component analysis (PCA) on the correlation matrix and Ward hierarchical clustering on Euclidean distances were applied to the standardised beverage means; with four beverages these analyses are descriptive, and a correlation required $|r| \geq 0.95$ for significance at $\alpha = 0.05$. Sensory associations between categories were tested with Pearson correlations for 20 panelists (critical $|r| = 0.444$), and the false discovery rate across the 40 coefficients was controlled with the Benjamini–Hochberg procedure. Paired attribute profiles were compared descriptively with the Wilcoxon signed-rank test, using attributes as paired units. The Monte Carlo simulation and the multivariate analyses were performed in Python 3.11 with NumPy 2.4, SciPy 1.17 and pandas 3.0.

3. Results
3.1. Microbial loads
Five of the eight microbial groups were detected in at least one beverage, and detected counts ranged from 1.30 (yeasts in rosé wine) to 3.00 (staphylococci in cashew wine) (Log_{10}CFU mL^{-1}) (Table 2). Faecal coliforms, moulds and pseudomonads were not detected (< 1.00 Log_{10}CFU mL^{-1}) in the beverages. Of the 32 combinations of beverage and microbial group interactions, 11 yielded microbial growth, and nine of those 11 microbial growths were estimated counts equivalent to fewer than 10 colonies per 0.1 mL plate. The artisanal beverages recorded 7 microbial group growth detections, with counts ranging from 1.48 to 3.00 (Log_{10}CFU mL^{-1}), whereas the commercial wines recorded 4 microbial group growth detections, with counts ranging from 1.30 to 1.90 (Log_{10}CFUmL^{-1}). Staphylococci were confined to the artisanal wine, which also carried the highest AAB count.

THBC ranged from 1.48 to 1.85 ($Log_{10}CFUmL^{-1}$) in the artisanal beverages and from not detected to 1.70 ($Log_{10}CFU\ mL^{-1}$) in the commercial red wines.

Cashew wine harboured the most diverse microbial load, comprising THBC (1.85 $Log_{10}CFU\ mL^{-1}$, the highest THBC value), LAB (1.60), AAB (1.70) and staphylococci (3.00), and its representative staphylococcal isolate was coagulase positive. Zobo recorded THBC (1.48), LAB (1.78) and the highest AAB count of the study 2.58 ($Log_{10}CFUmL^{-1}$). Rosé wine recorded yeasts (1.30), the highest LAB count (1.90) and AAB count (1.85), although THBC was not detected on nutrient agar. Red wine recorded only THBC (1.70 $Log_{10}CFUmL^{-1}$), and every other group were below the limit of detection (ND).

Table 2: Microbial loads ($log_{10}CFUmL^{-1}$) of the four beverages across eight microbial groups

Beverage	THBC	Faecal coliforms count	Yeasts count	Mould count	LAB count	AAB count	Pseudomonad count	Staphylococcal count
Cashew wine (artisanal)	1.85[e]	ND	ND	ND	1.60[e]	1.70[e]	ND	3.00
Zobo (artisanal)	1.48[e]	ND	ND	ND	1.78[e]	2.58	ND	ND
Red wine (commercial)	1.70[e]	ND	ND	ND	ND	ND	ND	ND
Rosé wine (commercial)	ND	ND						

Note. *THBC = total heterotrophic bacteria count; LAB = lactic acid bacteria; AAB = acetic acid bacteria; ND = not detected (< 1.00 log_{10} CFU mL^{-1}). [e] Estimated count equivalent to fewer than 10 colonies per 0.1 mL plate [25]. Staphylococci were enumerated on mannitol salt agar, and the representative isolate was coagulase positive. The staphylococcal count of 990 CFU mL^{-1} corresponds to 3.00 log_{10} CFU mL^{-1}.*

3.2. Hazard identification and exposure assessment

Overall range. Across the eight groups, back-transformed counts ranged from not detected to 990 $CFUmL^{-1}$, and only the staphylococci met the criteria for quantitative assessment (Table 3). Faecal coliforms, moulds and pseudomonads were absent from every beverage, whereas THB, yeasts, LAB and AAB represented hygiene, fermentative or spoilage functions rather than direct hazards.

Group range. The two potentially adverse findings, staphylococci (990 CFU mL^{-1}) and the highest AAB load (380 CFU mL^{-1}), both occurred in the artisanal beverages. In the commercial wines, no group exceeded 80 CFU mL^{-1}.

Individual beverage. The staphylococcal count in cashew wine fell within the marginal band of the FSANZ [27] criteria for coagulase-positive staphylococci (10^2 to < 10^3 CFU g^{-1}), only 10 CFU mL^{-1} below the unsatisfactory boundary. Because the concentration is fixed, the ingested viable dose scaled only with serving volume: 9.9×10^4 CFU for 100 mL, 1.5×10^5 CFU for 150 mL, 2.0×10^5 CFU for 200 mL and 2.5×10^5 CFU for 250 mL. For an intoxication, however, the relevant quantity is the concentration reached before consumption rather than the number of cells swallowed, and the measured concentration lay 2.0 log_{10} units below the 5.0 log_{10} CFU mL^{-1} threshold.

Table 3: Hazard classification of the enumerated microbial groups and their eligibility for quantitative microbial risk assessment

Microbial group	Count range (CFU mL^{-1})	Ecological role	Hazard	Carried to QMRA	Basis of assessment
Staphylococci (coagulase-positive)	ND to 990 (cashew wine)	Potential pathogen	Enterotoxin intoxication	Yes	FSANZ [27] criteria; threshold and growth model
Total heterotrophic bacteria	ND to 70 (cashew wine)	General microbiota	Hygiene indicator	No	Process hygiene
Faecal coliforms	ND in all	Faecal indicator	None detected	No	Absence satisfactory
Yeasts	ND to 20 (rosé wine)	Fermentative or spoilage	Quality	No	Product stability
Moulds	ND in all	Spoilage	None detected	No	Absence satisfactory
Lactic acid bacteria	ND to 80 (rosé wine)	Fermentative	Quality	No	Product stability
Acetic acid bacteria	ND to 380 (zobo)	Spoilage (acetification)	Quality	No	Product stability
Pseudomonads	ND in all	Spoilage or opportunistic	None detected	No	Absence satisfactory

Note. *QMRA = quantitative microbial risk assessment; ND = not detected (< 10 CFU mL^{-1}). Counts are back transformed from Table 2.*

3.3. Growth-coupled risk characterisation

At the measured pH of cashew wine (3.28 ± 0.04), the product pH lay below the minimum growth pH in every iteration, so the predicted growth rate was zero and no simulated serving reached the 5.0 or the 6.0 ($Log_{10}CFUmL^{-1}$) threshold at any holding time up to 48 h under any temperature scenario (Table 4; Figure 1B). Under the pH-permissive worst case (pH 7.0), the probability of reaching 5.0 $Log_{10}CFUmL^{-1}$ ranged from 0 to 100 (%) depending on temperature and holding time.

Under the pH-permissive worst case, ambient storage (24 to 34 °C) produced threshold exceedance in 5.5% of servings at 6 h, 28.4% at 8 h, 72.4% at 12 h and 97.5% at 18 h, with a median time to threshold of 9.7 h (5th percentile, 5.9 h). Room storage (18 to 26 °C) produced 5.6% at 12 h, 38.4% at 18 h and 66.5% at 24 h, with a median of 20.2 h (5th percentile, 11.8 h), whereas cool storage (4 to 10 °C) produced no exceedance within 48 h. When the threshold was raised to 6.0 $Log_{10}CFUmL^{-1}$, exceedance within 24 h fell to 98.3% for ambient storage (median, 12.8 h) and 40.4% for room storage (median, 26.4 h). In the deterministic pH-permissive curves (Fig. 1A), the time to 5.0 $Log_{10}CFUmL^{-1}$ increased from 5.3 h at 37 °C to 9.1 h at 30 °C, 14.8 h at 25 °C, 28.4 h at 20 °C, 75.0 h at 15 °C and 533.5 h at 10 °C, and no growth occurred at 4 °C.

Product pH controlled the outcome (Fig. 1C). For 24 h of ambient storage, exceedance was 0% at pH 4.1 or below, 3.3% at pH 4.2, 31.3% at pH 4.4, 49.7% at pH 4.5 and 98.6% at pH 5.0; for 24 h of room storage, the corresponding value at pH 5.0 was 46.3%. Within the pH-permissive ambient scenario, the time to threshold was most strongly associated with storage temperature (Spearman $\rho = -0.82$), followed by the reference growth rate ($\rho = -0.45$), the lag work term h_0 ($\rho = 0.31$), the initial concentration ($\rho = -0.09$) and the minimum growth temperature ($\rho = 0.04$).

Table 4: Monte Carlo probability (%) that a cashew wine serving reached 5.0 log_{10} CFU mL^{-1} of coagulase-positive staphylococci, by storage scenario, product pH and holding time

Storage scenario	Product pH	6 h	8 h	12 h	18 h	24 h	48 h	Median TTT (h)	5th percentile TTT (h)
Ambient (24 to 34 °C)	3.28 (measured)	0.0	0.0	0.0	0.0	0.0	0.0	Not reached	Not reached
Ambient (24 to 34 °C)	7.0 (worst case)	5.5	28.4	72.4	97.5	99.9	100.0	9.7	5.9
Room (18 to 26 °C)	3.28 (measured)	0.0	0.0	0.0	0.0	0.0	0.0	Not reached	Not reached
Room (18 to 26 °C)	7.0 (worst case)	0.0	0.1	5.6	38.4	66.5	99.6	20.2	11.8
Cool (4 to 10 °C)	3.28 (measured)	0.0	0.0	0.0	0.0	0.0	0.0	Not reached	Not reached
Cool (4 to 10 °C)	7.0 (worst case)	0.0	0.0	0.0	0.0	0.0	0.0	> 48	> 48

Note. *TTT = time to threshold; 50,000 iterations per scenario. Probabilities at 2 h and 4 h were 0.0% in every scenario.*

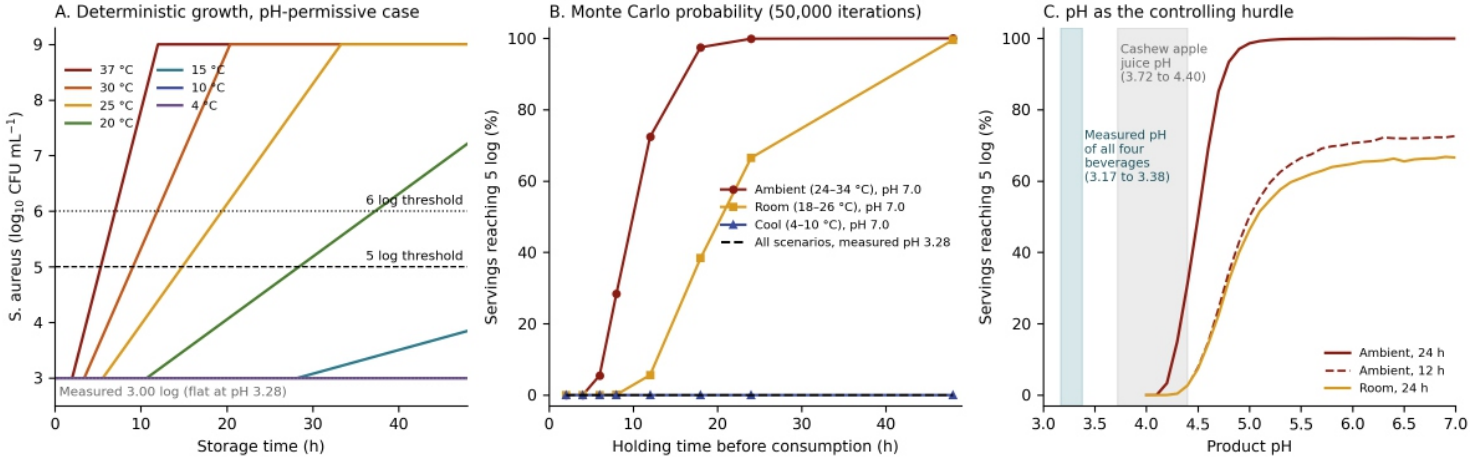

Figure 1. Growth-coupled quantitative microbial risk assessment of coagulase-positive staphylococci in cashew wine. (A) Deterministic growth from the measured 3.00 log_{10} CFU mL^{-1} at seven storage temperatures under the pH-permissive worst case (pH 7.0; T_min = 7.0 °C; lag of 2 h at 37 °C scaling inversely with growth rate); at the measured pH of 3.28, every curve remains flat. (B) Monte Carlo probability that a serving reached 5.0 log_{10} CFU mL^{-1} as a function of holding time under three temperature scenarios (50,000 iterations each). (C) Probability of reaching the threshold within 12 h or 24 h as a function of product pH; the shaded bands mark the measured pH range of the four beverages (3.17 to 3.38) and the pH range reported for cashew apple juice (3.72 to 4.40) [10].

3.4. Physicochemical properties

Across the four beverages, alcohol ranged from 0.00 to 6.84 (% v/v), total nitrogen from 0.07 to 0.17 (%), pH from 3.17 to 3.38, specific gravity from 1.00 to 1.07, soluble solids from 7.10 to 20.30 (°Brix), total solids from 2.59 to 17.22 (%) and TTA from 0.29 to 0.48 (%) (Table 5). The beverages differed significantly in alcohol, nitrogen, pH, soluble solids, total solids and TTA ($p \leq 0.020$), but not in specific gravity ($p > 0.05$) or viscosity ($p = 0.391$). Reconstruction from the summary statistics gave $F(3, 4) = 10.54$, $p = 0.023$ and $\eta^2 = .89$ for pH; $F(3, 4) = 2{,}195.68$, $p < 0.001$ and $\eta^2 > 0.99$ for total solids; and $F(3, 4) = 59{,}469.48$, $p < 0.001$ and $\eta^2 > 0.99$ for alcohol.

In the artisanal beverages, alcohol ranged from 0.00 (zobo) to 6.84 (cashew wine) (%), pH from 3.17 to 3.28, soluble solids from 8.10 to 20.30 (°Brix), total solids from 2.59 to 17.22 (%), TTA from 0.34 to 0.48 (%) and nitrogen from 0.10 to 0.17 (%). The commercial wines were compositionally closer to each other, with alcohol from 4.15 to 6.82 (%), pH from 3.36 to 3.38, soluble solids from 7.10 to 9.30 (°Brix), total solids from 3.88 to 6.94 (%), TTA from 0.29 to 0.48 (%) and nitrogen from 0.07 to 0.08 (%).

Cashew wine had the highest alcohol content (6.84 %, not different from red wine), the highest nitrogen (0.17%) and the lowest total solids (2.59%), and it shared the highest TTA (0.48%) with rosé wine. Red wine had the highest pH (3.38) and the lowest soluble solids (7.10 °Brix) and TTA (0.29%). Rosé wine was intermediate, with 4.15% alcohol, 9.30 °Brix and 6.94% total solids. Zobo contained no alcohol, the highest soluble solids (20.30 °Brix), total solids (17.22%) and specific gravity (1.07), and the lowest pH (3.17).

Six correlations were significant ($|r| \geq 0.96$, $p < 0.05$), and all involved soluble solids, total solids, specific gravity and alcohol, with alcohol correlating negatively with the other three (Figure. 2A). The first principal component explained 68.0% of the variance and was loaded by soluble solids (1.00), total solids (0.99), specific gravity (0.99), alcohol (−0.98) and pH (−0.85); the second explained 23.6% and was loaded by nitrogen (0.93), TTA (0.75) and pH (−0.46) (Figure 2B). Zobo scored +3.22 on the first component, whereas cashew wine (+1.63) and red wine (−1.51) occupied opposite ends of the second component, and rosé wine lay near the origin. Ward clustering joined red and rosé wine first (linkage distance 2.30), added cashew wine next (3.00) and joined zobo last (5.27).

Table 5: Physicochemical properties of the four beverages

Beverage	Alcohol (% v/v)	Total nitrogen (%)	pH	Specific gravity	Soluble solid (°Brix)	Total solids (%)	TTA (%)	Viscosity†
Cashew wine	6.84 ± 0.01^{a}	0.17 ± 0.01^{a}	3.28 ± 0.04^{ab}	1.00^{a}	8.10 ± 0.14^{c}	2.59 ± 0.23^{d}	0.48^{a}	0.14^{a}
Zobo	0.00^{c}	0.10^{b}	3.17 ± 0.04^{b}	1.07^{a}	20.30^{a}	17.22 ± 0.28^{a}	0.34^{b}	0.14^{a}
Red wine	6.82 ± 0.02^{a}	0.07^{c}	3.38 ± 0.01^{a}	1.00^{a}	7.10 ± 0.14^{d}	3.88 ± 0.15^{c}	0.29^{c}	0.14^{a}
Rosé wine	4.15 ± 0.03^{b}	0.08^{c}	3.36 ± 0.06^{a}	1.02^{a}	9.30 ± 0.14^{b}	6.94 ± 0.08^{b}	0.48 ± 0.01^{a}	0.14^{a}
p value	< 0.001	< 0.001	0.020	> 0.05	< 0.001	< 0.001	< 0.001	0.391

Note. *Values are means ± standard deviation of duplicate determinations; no standard deviation is shown where none was available. Within a column, means sharing a superscript letter did not differ significantly (Tukey's test, p > 0.05). TTA = total titratable acidity. The °Brix values of the wines represent apparent soluble solids. † Reported in the source data as Pa·s; the unit requires verification (Appendix A).*

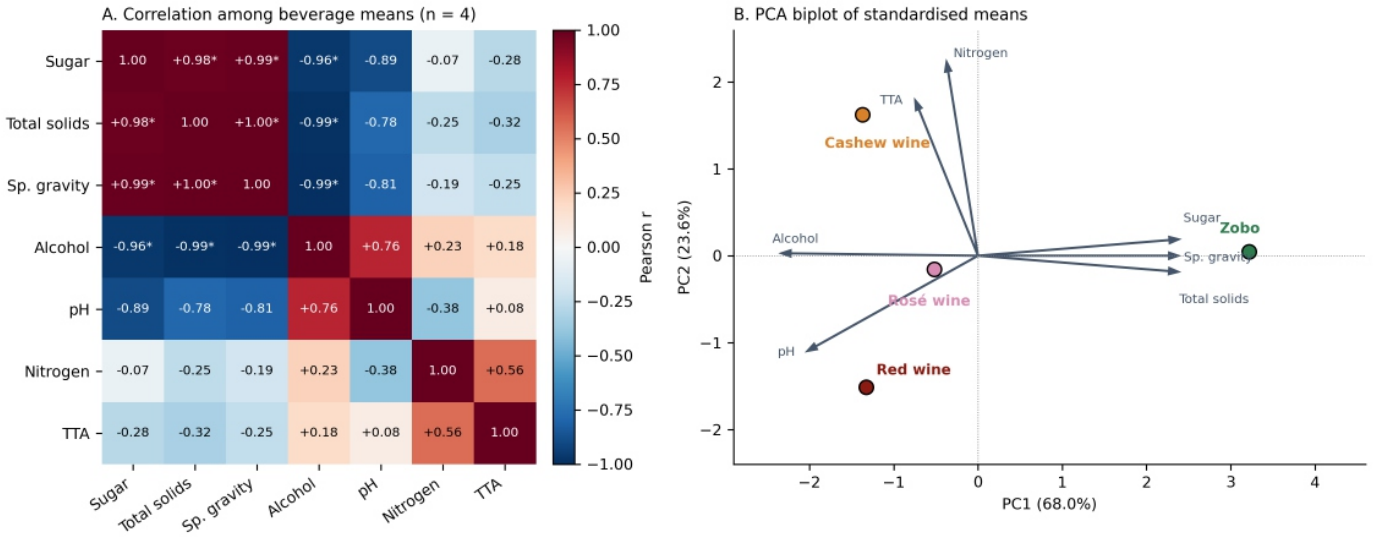

Figure 2. Multivariate structure of the physicochemical data in Table 5. (A) Pearson correlation matrix of the beverage means (n = 4); asterisks mark p < 0.05, which requires |r| ≥ 0.95. (B) Principal component analysis biplot of the standardised means (PC1 = 68.0%, PC2 = 23.6%). Viscosity was excluded because it did not vary. Both panels are descriptive because each beverage contributes a single mean.

3.5. Sensory profiles

Attribute means ranged from 1.3 (creaminess of rosé wine) to 4.8 (creaminess of cashew wine) on the five-point scale (Figure 3), and category means ranged from 1.90 (olfactory score of rosé wine) to 4.30 (mouthfeel score of cashew wine) (Figure 4). Scores for overall impression varied least, from 3.10 to 3.35. The attribute means for the artisanal beverages ranged from 2.1 to 4.8, with grand means of 3.12 for cashew wine and 3.19 for zobo, whereas those for the commercial wines ranged from 1.3 to 4.0, with grand means of 2.68 for rosé wine and 2.66 for red wine. The artisanal beverages scored higher in the olfactory category (2.87 to 3.07 against 1.90 to 2.23) and in the mouthfeel category (3.05 to 4.30 against 2.35 to 2.55).

Cashew wine was characterised by creaminess (4.8), aroma intensity (3.8) and smoothness (3.8), and it scored lowest for sweetness and herbal aroma (both 2.1); it exceeded rosé wine on eight attributes, scored lower on three and equaled it on one (Wilcoxon signed-rank test, p = 0.21).

Rosé wine received the highest overall quality score of the study (4.0) together with high sweetness (3.7), but low creaminess (1.3), herbal aroma (1.4) and fruity aroma (1.5). Zobo scored highest for colour intensity (3.9), aroma intensity (3.8) and overall quality (3.7), and it exceeded red wine on 10 of 13 attributes, equaled it for acidity (3.5) and typicity (2.8) and scored lower only for clarity (2.5 against 2.8) (p = 0.002). Red wine was defined by acidity (3.5), overall quality (3.4) and aroma intensity (3.3), and its fruity and floral aromas were the weakest in its session (both 1.7). Twelve of the 40 inter-category correlations were significant at p < 0.05, and six remained significant after false discovery rate control (Table 6). In zobo, mouthfeel and visual scores correlated strongly with overall impression (r = 0.81 and r = 0.71) and with each other (r = 0.81). Visual and olfactory scores correlated in red wine (r = 0.80), olfactory score correlated with overall impression in cashew wine (r = 0.58), and mouthfeel correlated negatively with gustatory score in rosé wine (r = −0.59).

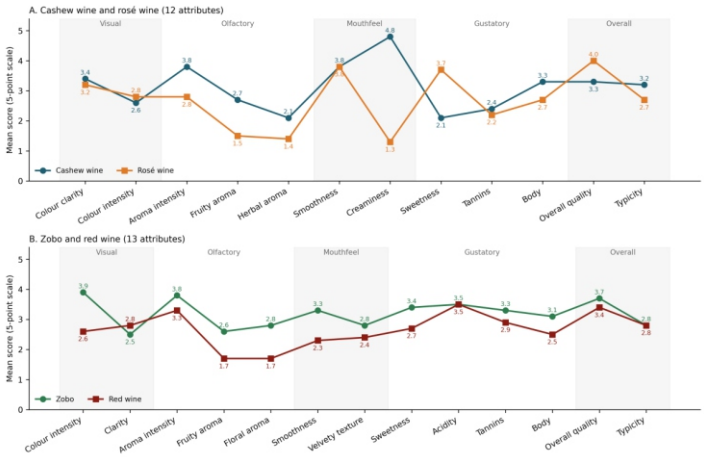

Figure 3. Mean sensory scores of the four beverages on a five-point intensity scale (20 panelists). (A) Cashew wine and rosé wine across 12 attributes. (B) Zobo and red wine across 13 attributes, including acidity. Shaded bands delimit the five attribute categories.

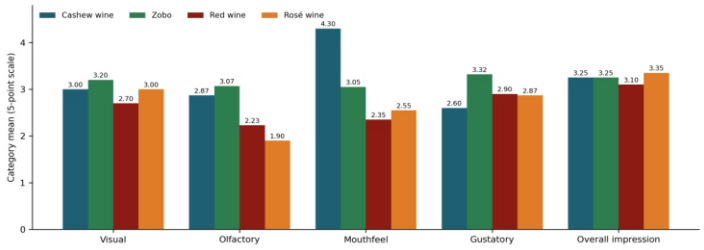

Figure 4. Sensory category means of the four beverages, calculated as the arithmetic means of the attribute means in Figure 3. The gustatory mean for zobo and red wine includes acidity, which was not scored for cashew wine and rosé wine.

Table 6: Pearson correlation coefficients (r) between sensory attribute categories, by beverage (n = 20 panelists)

Category pair	Cashew wine	Zobo	Red wine	Rosé wine
Visual and olfactory	0.28	0.16	0.80*†	0.02
Visual and mouthfeel	0.48*	0.81*†	0.48*	0.21
Visual and gustatory	−0.17	0.12	0.00	−0.15
Visual and overall impression	0.27	0.71*†	−0.24	−0.04
Olfactory and mouthfeel	0.14	0.31	0.48*	0.11
Olfactory and gustatory	−0.23	−0.44	0.00	0.20
Olfactory and overall impression	0.58*†	0.22	−0.24	0.22
Mouthfeel and gustatory	0.03	0.08	−0.15	−0.59*†
Mouthfeel and overall impression	0.56*	0.81*†	0.24	−0.50*
Gustatory and overall impression	0.15	−0.32	0.11	0.50*

Note. ** p < .05 (critical |r| = .444, df = 18). † Significant after Benjamini–Hochberg control of the false discovery rate across all 40 coefficients (q < .05). The identical coefficients of the visual and olfactory categories with the remaining categories in red wine require verification against the panel data (Appendix A).*

4. Discussion

4.1. Microbial quality and hygiene status

The absence of faecal coliforms, moulds and pseudomonads from all four beverages indicates that gross faecal contamination and heavy environmental spoilage were not present in the sampled lots. This agrees with Nwaiwu et al. [1], who found fewer hygiene indicators in alcoholic than in non-alcoholic Nigerian beverages, and it is consistent with the strong acidity of every sample (pH 3.17 to 3.38). The zobo examined here, however, departs from much of the Nigerian literature. Okoi et al. [3] recovered *S. aureus*, *E. coli*, *Salmonella* and *Klebsiella* from street-hawked zobo at counts in the order of 10^3 CFUmL^{-1}, and Okolie et al. [4] reported up to 7.2×10^5 CFUmL^{-1} after six weeks of storage. Two explanations, which are not mutually exclusive, may account for the difference. On the one hand, the zobo in this study was collected from producers in sealed bottles, so the handling and dispensing steps that Okoi et al. [3] identified as contamination routes were avoided. On the other hand, three bottles of one product cannot represent an unregulated market, and nine of the eleven positive counts were estimated counts derived from fewer than 10 colonies per plate, which carry a wide relative uncertainty [25]. The low loads should therefore be read as the status of the sampled lots rather than as evidence that zobo is generally safe.

LAB and AAB counts were the most frequently detected groups, and their distribution reflects fermentation ecology as much as contamination. LAB (1.60 to 1.90 $\log_{10}$ CFU mL^{-1}) occurred in cashew wine, zobo and rosé wine. The finding is in agreement with Adegbehingbe et al. [8] report of isolation of LAB from zobo that inhibited *S. aureus* and *E. coli*, and Yehuala et al. [32] showed that selected LAB isolates from a traditional Ethiopian beverage tolerated 8% to 10% ethanol, which explains their persistence in wines containing 4.15% to 6.84% alcohol. Nonetheless, LAB are not uniformly beneficial in wine, because they are the main producers of histamine and tyramine during winemaking [33]. The AAB count in zobo (2.58 $\log_{10}$ CFU mL^{-1}) exceeded those in the wines by 0.73 to 0.88 log units. The finding collaborates with Mitina et al. [34], that the AAB content of mature wine explained 33.7% of the variance in volatile acidity, and Parra et al. [35] described excessive acetic acid production by AAB as a major challenge that leads to consumer rejection of wine. Because zobo is unfermented and sugar-rich (20.30 °Brix), its AAB population more probably reflects contamination after preparation and incipient acetification than a residual fermentation flora. Conversely, AAB are poorly controlled by some antimicrobial treatments used in winemaking, such as chitosan [36], so their presence in rosé wine (1.85 $\log_{10}$ CFU mL^{-1}) does not by itself imply poor hygiene.

The yeast count in rosé wine and the absence of THB on nutrient agar in the same wine, despite LAB and AAB on selective media, also deserve comment. The findings of this study collaborate with the report of low yeast numbers in a bottled commercial wine which are compatible with incomplete stabilisation, because sulphur dioxide and alternative non-thermal treatments are applied precisely to prevent yeast spoilage during wine storage [37]. The discrepancy between the THB and selective counts is methodological, because aerobic incubation on nutrient agar at 37 °C does not favour acid-adapted LAB and AAB; THB alone therefore underestimates the viable load of acidic beverages. Red wine, by contrast, contained only THB (1.70 $\log_{10}$CFUmL^{-1}), which suggests a well-stabilised product. Commercial status thus did not guarantee microbiological uniformity, a conclusion that parallels the compositional variability of Nigerian red wines reported by Ofoedu et al. [12].

4.2. Staphylococcal risk: contamination without growth

The detection of coagulase-positive staphylococci at 990 CFU mL^{-1} in cashew wine is the principal safety finding. Against the FSANZ [27] criteria, the count is marginal and lies only 10 CFU mL^{-1} below the unsatisfactory boundary. Its presence in a product at pH 3.28 containing 6.84% ethanol is best explained by contamination after fermentation, through handling, utensils or bottling, rather than by growth in the finished wine. This interpretation is consistent with Okoye and Eruteya [16], who classified 68 of 100 ready-to-eat foods from Obio/Akpor, Rivers State, as borderline or worse for *S. aureus* and detected the enterotoxin A gene in nine isolates. Local handling practices therefore remain a credible source of enterotoxigenic strains.

The growth-coupled QMRA shows that the count does not by itself translate into a toxin risk. At the measured pH, no simulated serving reached the toxigenic threshold under any storage scenario, because the product pH lay 0.7 to 1.2 units below the minimum growth pH range used in the model [22]. Evidence from other beverages supports this outcome. Çobo et al. [23] observed pathogen growth in non-alcoholic beer but not in a low-alcohol beer of 3.2% (v/v), and Maust et al. [24] identified pH as the decisive hurdle in a non-alcoholic beer matrix; cashew wine combined a lower pH with roughly twice that ethanol concentration. In zobo, which had the lowest pH (3.17) and no detectable staphylococci, the organic acids of the calyces [6] and plant polyphenols with anti-staphylococcal activity [38] would add further hurdles.

The present model also differs from a temperature-only screening scenario, which ignored pH, implied growth at 4 °C and applied a fixed 2 h lag at every temperature. Regression of the growth rates of that scenario yielded a notional minimum growth temperature of about 1.3 °C, which conflicts with published kinetics: Bai et al. [28] estimated 6.85 °C in raw pork, Jia et al. [21] reported 5.0 to 7.2 °C across dairy matrices, and Flores et al. [19] observed no growth at 7 °C over 72 h in mechanically separated chicken meat. With these limits and a lag that lengthens as growth slows, even the pH-permissive, worst case produced lower and later exceedance than the screening scenario, namely 72.4% of servings at 12 h under ambient storage and 5.6% at 12 h under room storage, compared with approximately 100% and 47% in the screening scenario. The sensitivity analysis further showed that storage temperature dominated the time to threshold ($\rho = -0.82$), and cool storage prevented exceedance within 48 h. Refrigeration would therefore become the critical control only if the acidity of the product were compromised.

Several considerations nevertheless argue against complacency. First, the staphylococci survived in the wine long enough to be enumerated, and *S. aureus* is notably tolerant of adverse conditions, persisting above 3.8 log CFU g^{-1} for 30 d in brined cheese at 4 °C [22]. Second, staphylococcal enterotoxins resist heat and proteolysis, tolerate a wide pH range [13] and may remain in food after the producing cells have declined [39]. If the organism had grown in cashew apple juice before fermentation acidified it, toxin could be present without a high count at sampling. The pH scan quantifies this possibility: at the upper pH reported for cashew apple juice (4.40) [10], 31.3% of servings would exceed the threshold within 24 h of ambient holding, whereas at pH 4.1 or below the probability was zero. Against this scenario, Haddad et al. [22] reported that enterotoxin production requires a pH of 5.3 to 7.0, which argues against toxin formation even in juice at pH 4.4. Third, the 5.0 $\log_{10}$ CFU mL^{-1} threshold adopted here is conventional [17],

while recent experiments detected enterotoxins only at about 6 log CFU mL^{-1} [18] or at 6.5 to 8.0 log CFU g^{-1} [19]. The primary threshold is therefore precautionary, which is appropriate given that enterotoxins act in nanogram amounts [14] and were found in 27.11% of contaminated mixed and ready-to-eat foods [15]. Finally, the model omitted both inactivation at sub-minimal pH and the inhibitory effect of ethanol, and its minimum temperature and pH distributions were drawn from other matrices; each of these choices makes the estimates conservative rather than optimistic. Confirmatory testing of cashew wine for enterotoxins by the reference method EN ISO 19020, which Schaarschmidt et al. [14] described as the replacement for the former European screening method, would resolve the remaining uncertainty.

4.3. Physicochemical differentiation of artisanal and commercial beverages

The alcohol content of cashew wine (6.84%) did not differ from that of red wine (6.82%), whereas rosé wine contained 4.15%. The red wine value is far below the 14.44% to 15.21% reported for imported red wines sold in Nigeria by Ofoedu et al. [12], which suggests that the commercial red wine examined here belongs to a low-alcohol category rather than to conventional table wine. All four beverages were strongly acidic, and the pH of the commercial wines (3.36 to 3.38) was lower than that of the domestic red wines (4.03 to 4.16) analysed by Ofoedu et al. [12]. This acidity favours microbiological stability, as discussed above. By contrast, zobo (pH 3.17) was less acidic than the products described by Okolie et al. [4] (pH 2.06 to 2.79) and Adisa et al. [5] (pH 2.8), a difference that may reflect the calyx-to-water ratio or the proportion of added sugar.

Zobo contained 20.30 °Brix of soluble solids, more than three times the 3 to 6 °Brix reported for mulled zobo [4], which indicates heavy sweetening. The soluble solids of the wines (7.10 to 9.30 °Brix) were comparable to the 6.98 to 8.04 °Brix of imported red wines [12], but these values must be interpreted as apparent soluble solids, because the refractive index of wine responds to sugars, tartaric acid, glycerol and ethanol, not to sugars alone [31]. One further anomaly deserves comment. The soluble solids of zobo (20.30 °Brix) exceeded its total solids (17.22%), which cannot occur when both are expressed on the same mass basis; different expression bases or losses of heat-labile sugars during oven drying are possible explanations. The TTA of the wines (0.29% to 0.48%) was lower than the 0.76% to 0.83% of imported red wines [12].

The multivariate analyses condensed these differences into a fermentation axis. PC1 (68.0%) contrasted soluble solids, total solids and specific gravity with alcohol, thereby separating unfermented, sugar-rich zobo from the three wines, whereas PC2 (23.6%) separated nitrogen-rich and acidic cashew wine from red wine. Ward clustering joined the two commercial wines first, which supports the artisanal versus commercial framing of the study. These results must nevertheless be read descriptively. With four beverages, a correlation requires $|r| \geq .95$ to reach significance, and the six significant correlations were driven largely by zobo; for example, the negative correlation between soluble solids and alcohol fell from −.96 to −.89 when zobo was removed. The correlation structure therefore describes the contrast between fermented and unfermented products rather than general relationships among the variables.

4.4. Sensory profiles and their compositional basis

The artisanal beverages achieved higher grand means (3.12 and 3.19) than the commercial wines (2.66 and 2.68), chiefly because of their olfactory and mouthfeel scores. Zobo equaled or exceeded red wine on 12 of 13 attributes, and its high colour intensity (3.9) is consistent with the anthocyanin-rich calyces from which it is prepared [40]. Its sweetness score (3.4), however, was lower than that of rosé wine (3.7) despite more than twice the soluble solids, although the two beverages were scored in different sessions. The organic acids of *H. sabdariffa* [6], reflected in the acidity score of 3.5 and the pH of 3.17, probably suppressed perceived sweetness. Cashew wine was distinguished by creaminess (4.8) and aroma intensity (3.8) together with the lowest sweetness (2.1), which agrees with its low soluble solids (8.10 °Brix). Glycerol and phenolic compounds formed during cashew wine fermentation influence its sensory character [11], and the tannins of the cashew apple [9,10] may have contributed to its distinctive mouthfeel. Notably, the two beverages with the highest sensory scores were also those with the highest microbial loads, so consumer preference is likely to increase exposure to products whose hygiene after fermentation is not regulated.

The correlations between categories suggest different drivers of overall impression. In zobo, overall impression was associated with mouthfeel ($r = 0.81$) and appearance ($r = 0.71$), whereas in cashew wine it was associated with aroma ($r = 0.58$). Rosé wine received the highest overall quality score (4.0) despite weak aroma, and its gustatory score correlated positively with overall impression ($r = 0.50$), although this coefficient did not survive false discovery rate control. These patterns are consistent with sweetness and texture, rather than aromatic complexity, driving acceptance among untrained consumers, but the evidence is limited. The panel was untrained and small; the two sessions used different attribute sets, so cross-session comparisons such as cashew wine against zobo are only indicative; and the Wilcoxon tests treated attributes as paired units and are therefore descriptive.

5. Conclusion

Artisanal beverages (cashew wine and zobo) housed more microbial loads than the commercial wines, including coagulase-positive staphylococci in cashew wine, yet their acidity (pH 3.17 to 3.28) prevented any predicted staphylococcal growth. Safety therefore depends on preserving low pH and preventing contamination after fermentation. However, routine enterotoxin testing, hygienic bottling and pH monitoring are strongly recommended for artisanal beverages marketed in Nigeria.

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