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A Palm Wine-Derived Yeast Consortium Overcomes the Ethanol-Aroma Trade-off in Honey Mead Fermentation

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ABSTRACT

Background: mead remains an underexploited fermented beverage in sub-Saharan Africa despite the regional abundance of honey, and commercial *Saccharomyces cerevisiae* strains often yield meads that lack aromatic complexity and regional identity. Palm wine harbours a diverse indigenous yeast community that has rarely been evaluated as a fermentation resource for honey must.
Objective: to isolate and molecularly identify yeast species from palm wine and to compare their fermentation performance, physicochemical quality and sensory acceptability, singly and as a mixed consortium, during honey must fermentation.
Methods: yeasts isolated from *Elaeis guineensis* and *Raphia hookeri* palm wine were identified by lactophenol cotton blue staining and internal transcribed spacer (ITS) sequencing. Honey must (16 to 20 °Brix) was fermented for 14 days at 30 °C with *Saccharomyces cerevisiae* strain YCH497, *Meyerozyma guilliermondii* strain L11-2, *Candida rugosa* strain UY, and a three-species consortium, followed by 30 days of maturation. Fermentation kinetics, must-Brix and temperature optimisation, matured-wine physicochemistry, sensory quality (nine-point hedonic scale, n = 10) and bacteriological safety were assessed by one-way ANOVA with Tukey or Duncan post-hoc separation.
Results: the consortium fermented fastest and most completely, reaching 12.22 ± 0.02% alcohol by volume (ABV) and 0.25 °Brix residual sugar by day 14, against 11.16 ± 0.02% ABV for S. cerevisiae and 8.27% and 6.82% ABV for C. rugosa and M. guilliermondii respectively (all $p < 0.001$). This ranking held across must-Brix (10 to 20 °Brix) and temperature (10 to 30 °C) optimisation. Contrary to the conventional trade-off between fermentation vigour and sensory complexity, the consortium also received the highest sensory scores for colour, aroma, taste, mouthfeel and overall acceptability (8.7 ± 0.48 of 9), and matured wines from every treatment were free of coliforms, Escherichia coli, Staphylococcus aureus and Salmonella spp.
Conclusion: a three-species consortium of palm wine-derived yeasts simultaneously maximised fermentation efficiency and sensory quality of honey mead, indicating that palm wine is an underused reservoir of complementary indigenous yeasts for value-added mead production in tropical honey-producing regions.
Keywords: mead; honey must fermentation; palm wine yeast; yeast consortium; Meyerozyma guilliermondii; non-Saccharomyces yeast

1. INTRODUCTION

Fermented beverages occupy a distinctive place among humanity's oldest biotechnologies, and mead, produced by fermenting diluted honey with yeast, is widely considered the earliest of them, predating both grape wine and beer [1]. Archaeological residues from Neolithic China and textual references from the Rigveda, classical Greece and Norse tradition attest to a continuous, cross-cultural relationship between honey fermentation and human ritual, medicine and commerce [2],[3]. Despite this deep heritage and the renewed 21st-century interest in artisanal mead [4],[5], production in sub-Saharan Africa remains marginal relative to the region's honey supply. In Nigeria, where honey is abundant and culturally significant, mead has attracted little systematic scientific investment, and the fermentation resources that could underpin a local mead industry remain largely unexamined.

The outcome of any mead fermentation is determined chiefly by the yeast strain used. Commercial *Saccharomyces cerevisiae* dominates industrial practice because of its high ethanol tolerance and predictable kinetics [6], but honey must is an unusual substrate: highly concentrated in sugar, low in assimilable nitrogen and acidic, conditions that constrain yeast physiology in ways that differ from grape or cereal fermentation [7]. Under these conditions, standard commercial strains frequently produce meads that ferment reliably but lack aromatic depth and any sense of regional identity [8]. Non-*Saccharomyces* yeasts such as *Pichia*, *Candida* and *Hanseniaspora* species are known to enrich aroma and flavour through ester and higher-alcohol production, but this sensory benefit is generally reported alongside slower, less complete fermentation and lower ethanol tolerance [9],[10]. This apparent trade-off between fermentation vigour and sensory complexity has shaped much of the applied literature on alternative starter cultures for wine, beer and mead alike [11].

Palm wine, a spontaneously fermented sap of *Elaeis guineensis* and *Raphia hookeri* widely consumed across West and Central Africa, harbours a microbial community that includes *Saccharomyces*, *Candida*, *Pichia* and *Hanseniaspora* species adapted to high-sugar, low-pH, tropical fermentation niches [12],[13]. These indigenous strains have been proposed as a reservoir of fermentation traits, including osmotolerance, acid resistance and distinctive ester production, that could diversify and improve locally produced alcoholic beverages [14],[15]. Nigeria offers a natural opportunity to pair two abundant, culturally embedded resources, honey and palm wine, yet no study has systematically evaluated palm wine-derived yeasts as starters for honey must fermentation, nor tested whether the ethanol-yield and aroma-complexity strengths of different species can be captured simultaneously through mixed inoculation.

Mixed and consortium fermentations have improved kinetics and product complexity in other beverage systems through metabolic complementarity and cross-feeding among co-fermenting species [16],[17], but this strategy has not been tested for honey must using an entirely palm wine-derived starter set. We hypothesised that a consortium combining a palm wine *Saccharomyces cerevisiae* isolate with two non-*Saccharomyces* isolates would match or exceed single-strain *S. cerevisiae* fermentation kinetics while also improving sensory quality, thereby resolving the conventional trade-off between fermentation efficiency and aroma complexity. This study isolated and molecularly identified yeast species from palm wine and evaluated their single-strain and consortium fermentation performance, must-Brix and temperature optimisation, matured-wine physicochemistry, sensory acceptability and bacteriological safety in honey must, with the aim of establishing whether native yeast consortia can convert two underused regional bioresources into a reproducible, quality-assured mead.

2. Materials and Methods

2.1 Sample collection and study area

Palm wine was collected between 06:00 and 08:00 from *Elaeis guineensis* and *Raphia hookeri* trees in Ugwuomu Nike, Enugu State, Nigeria, directly from tapping receptacles into sterile 1 L amber bottles, transported on ice at 4 °C and processed within 12 h. Raw, unadulterated honey was obtained from certified beekeepers in Opi Nsukka, Enugu State, and stored in sterile, airtight, food-grade containers at 25 ± 2 °C in the dark until use [18]. All laboratory work was carried out in the Department of Applied Microbiology, Enugu State University of Science and Technology, Nigeria.

2.2 Isolation and identification of yeasts

Diluted palm wine (0.1 mL) was spread onto Yeast Extract Peptone Dextrose (YEPD) agar supplemented with 100 mg/L chloramphenicol and incubated aerobically at 28 °C for 48 h [19]. Presumptive isolates were characterised by colony morphology and by lactophenol cotton blue staining examined under ×10 and ×40 objectives [20]. Genomic DNA was extracted with a ZR Fungal/Bacterial DNA MiniPrep kit, and the internal transcribed spacer (ITS) region was amplified with primers ITS1 and ITS4 (initial denaturation 94 °C/5 min; 36 cycles of 94 °C/30 s, 50 °C/30 s, 72 °C/45 s; final extension 72 °C/7 min). Amplicons were sequenced on an Applied Biosystems 3130xl Genetic Analyzer using the BigDye Terminator v3.1 kit, and sequences were aligned and analysed in BioEdit and MEGA X against GenBank references by BLAST.

2.3 Honey substrate characterisation and must preparation

Moisture, ash, crude fibre, protein (Kjeldahl), free and lactonic acidity, total solids, sugar content, electrical conductivity and pH of the honey were determined following AOAC [21] and Aniaku and Ogunbodede [19]. Honey must was aseptically diluted to 16 to 20 °Brix, adjusted to pH 3.5 with food-grade citric acid, supplemented with diammonium phosphate (0.3 to 0.5 g/L) and pasteurised at 70 °C for 1 min before inoculation.

2.4 Inoculum standardisation, fermentation and maturation

Each isolate was activated in YEPD broth (28 ± 2 °C, 24 h) and standardised to an optical density of 0.5, with viable counts confirmed by spread-plating on YEPD agar following standard inoculum-preparation protocols [22]. Dried yeast biomass, prepared by shake-flask propagation and centrifugation [23], was inoculated at 5 g per litre of must into four treatments: *Saccharomyces cerevisiae* strain YCH497 (SC), *Meyerozyma guilliermondii* strain L11-2 (MG), *Candida rugosa* strain UY (CR) and an equal-proportion three-species consortium (CONS). Primary fermentation ran for 14 days at 30 °C in 1.5 L glass fermenters, after which lees were removed. The virgin wine was matured at 10 °C for 30 days with 10 g/L sucrose (krausening) to promote esterification, 5 g/L bentonite for clarification and 2.0 g/L potassium metabisulfite as preservative.

2.5 Optimisation of must sugar concentration and fermentation temperature

The influence of initial must concentration was tested at 10, 15 and 20 °Brix at 30 °C, and the influence of temperature was tested at 10, 25 and 30 °C at a fixed 0.5% (w/v) inoculum concentration, for each of the four treatments, with fermentation and maturation performed as above.

2.6 Analytical, sensory and microbiological methods

Specific gravity was monitored by hydrometry at 48 h intervals [24],[25]; alcohol by volume (ABV) by alcoholmetry [26]; residual sugar by refractometry [19]; pH by calibrated pH meter; and total acidity by titration to pH 8.30 with 0.1 M NaOH, expressed as acetic acid equivalents. Yeast populations were enumerated on YPD agar at days 0, 2, 4, 7 and 14 by standard plate count and expressed as log10 CFU/mL. Turbidity of matured wine was measured nephelometrically. Sensory quality was assessed by a ten-member panel of trained and untrained assessors using a nine-point hedonic scale (1, dislike extremely; 9, like extremely) for colour, aroma, taste, mouthfeel and overall acceptability, under coded, randomised presentation [27],[28]. Bacteriological quality of matured wines was assessed by serial dilution and plating on Nutrient, MacConkey and Salmonella-Shigella agars, with isolates characterised by Gram reaction and standard biochemical tests (catalase, coagulase, citrate, indole, oxidase, methyl red and sugar fermentation).

2.7 Statistical analysis

Data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to test the effect of yeast treatment, fermentation day, must-Brix and temperature on fermentation and quality parameters, with significance set at $p < 0.05$. Where main or interaction effects were significant, means were separated by Tukey's honestly significant difference (HSD) test or Duncan's multiple range test (DMRT). Analyses were performed in SPSS version 25 and GraphPad Prism 9.

3. RESULTS

3.1 Isolation and molecular identification of palm wine yeasts

Three morphologically distinct yeasts were recovered from palm wine. Isolate PW1 formed creamy, smooth, pasty colonies with oval budding cells and occasional pseudohyphae; PW2 formed creamy, smooth to mucoid colonies with oval, multipolar-budding cells; and PW3 formed creamy to slightly pink, smooth to farinose colonies with oval to elongated budding cells and pseudohyphae. Amplification of the ITS region yielded a single 650 bp product for all three isolates (Figure 1). BLAST comparison against GenBank identified PW1 as *Candida rugosa* strain UY (98.55% similarity to accession JQ974952.1), PW2 as *Saccharomyces cerevisiae* strain YCH497 (92.14% similarity to KM982975.1) and PW3 as *Meyerozyma guilliermondii* strain L11-2 (99.11% similarity to DQ683005.1), confirming the morphological presumptive identifications and establishing that palm wine yielded one *Saccharomyces* and two non-*Saccharomyces* species suitable for fermentation trials.

3.2 Physicochemical quality of the honey substrate

The pasteurised honey met standard compositional benchmarks for a mature, low-moisture blossom honey (Table 1): moisture 17.40 ± 0.10%, total solids 82.53 ± 0.15%, sugar (glucose plus fructose) 69.27 ± 0.17%, total acidity 38.03 ± 0.25 meq/kg and pH 3.93 ± 0.06. Moisture below the Codex Alimentarius limit of 20% and the correspondingly high total solids indicated a substrate that was microbiologically stable and fermentable once diluted, with a moderate electrical conductivity (0.43 ± 0.01 mS/cm) consistent with a floral rather than honeydew origin.

Table 1. Physicochemical properties of the pasteurised honey substrate

Parameter	Value
Free acidity (meq/kg)	32.83 ± 0.25
Lactonic acidity (meq/kg)	8.20 ± 0.10
Total acidity (meq/kg)	38.03 ± 0.25
Moisture content (%)	17.40 ± 0.10
Protein (mg/g)	1.96 ± 0.02
Mineral content (mg/100 g)	45.93 ± 0.25
Ash content (%)	0.21 ± 0.01
Electrical conductivity (mS/cm)	0.43 ± 0.01
Sugar content, glucose + fructose (%)	69.27 ± 0.17
Ph	3.93 ± 0.06
Total solids (%)	82.53 ± 0.15
Specific gravity	1.41 ± 0.01

Values are mean ± SD of triplicate determinations.

3.3 Fermentation kinetics of honey must

All four treatments began fermentation from statistically indistinguishable starting conditions (specific gravity [SG] ≈ 1.083, pH 3.50 ± 0.02, 20 °Brix, $p > 0.44$ for all day-0 comparisons), confirming a common baseline. From day 2 onward the treatments diverged significantly and remained distinct for the remainder of fermentation (ANOVA $p < 0.001$ for treatment, day and their interaction across all parameters). The three-species consortium (CONS) consistently fermented fastest and most completely: SG fell from 1.05116 ± 0.00046 on day 2 to 0.98942 ± 0.00051 on day 14, residual sugar fell to 0.25 °Brix, ABV reached 12.22 ± 0.02% and total acidity reached 4.01 ± 0.06 g/L by day 14. *Saccharomyces cerevisiae* (SC) followed a similar trajectory (final ABV 11.16 ± 0.02%, residual sugar 0.50 °Brix), while *Candida rugosa* (CR) and *Meyerozyma guilliermondii* (MG) fermented more slowly and incompletely, reaching only 8.27 ± 0.02% and 6.82 ± 0.02% ABV, with 6.20 and 8.00 °Brix residual sugar remaining, respectively. Yeast populations rose fastest in CONS and SC during the first four days (peaking at 8.556 ± 0.073 and 8.518 ± 0.067 log10 CFU/mL), then declined as ethanol and organic acids accumulated, whereas MG and CR maintained comparatively higher late-fermentation viability (7.474 ± 0.071 and 7.444 ± 0.063 log10 CFU/mL at day 14), consistent with slower substrate utilisation and lower stress exposure. Across every measured variable the fermentation-efficiency ranking was consistent: CONS > SC > CR > MG (Tukey HSD, $p < 0.05$ for all pairwise comparisons).

3.4 Effect of initial must sugar concentration on fermentation performance

Increasing must concentration from 10 to 20 °Brix significantly increased ABV in every treatment ($p < 0.001$), but the magnitude of the response depended strongly on organism identity (organism × °Brix interaction, $p < 0.001$; Table 2). SC and CONS converted the additional sugar efficiently, with ABV rising from 5.96% to 11.40% and from 6.16% to 11.66% respectively, while residual sugar and final SG remained low across all °Brix levels. MG and CR converted sugar far less efficiently: residual sugar rose from 3.7 to 9.0 °Brix (MG) and 3.8 to 9.6 °Brix (CR), final SG rose to 1.029 and 1.032 at 20 °Brix, and acetic acid production (1.10 to 1.20 g/L) was two- to threefold higher than in SC or CONS (0.30 to 0.45 g/L) at every °Brix level tested (all $p < 0.001$).

Table 2: Effect of initial must sugar concentration (°Brix) on fermentation outcomes

Treatment	Must (°B)	Final SG	ABV (%v/v)	Residual sugar (°Brix)	pH	Total acidity (g/L acetic)
MG	10	1.009 ± 0.002	4.08 ± 0.20	3.7 ± 0.3	3.50 ± 0.02	0.65 ± 0.05
MG	15	1.017 ± 0.002	5.83 ± 0.20	6.0 ± 0.3	3.48 ± 0.05	0.85 ± 0.05
MG	20	1.029 ± 0.002	7.13 ± 0.20	9.0 ± 0.2	3.46 ± 0.02	1.10 ± 0.05
CR	10	1.009 ± 0.002	4.02 ± 0.20	3.8 ± 0.3	3.47 ± 0.02	0.70 ± 0.01
CR	15	1.018 ± 0.002	5.64 ± 0.20	6.3 ± 0.3	3.45 ± 0.02	0.95 ± 0.05
CR	20	1.032 ± 0.002	6.74 ± 0.20	9.6 ± 0.4	3.43 ± 0.02	1.20 ± 0.05
SC	10	0.995 ± 0.002	5.96 ± 0.20	0.8 ± 0.3	3.36 ± 0.02	0.35 ± 0.05
SC	15	0.994 ± 0.002	8.75 ± 0.20	1.5 ± 0.3	3.32 ± 0.02	0.40 ± 0.05
SC	20	0.996 ± 0.002	11.40 ± 0.20	2.4 ± 0.3	3.28 ± 0.02	0.45 ± 0.05
CONS	10	0.993 ± 0.002	6.16 ± 0.20	0.5 ± 0.3	3.40 ± 0.02	0.30 ± 0.05
CONS	15	0.993 ± 0.002	8.94 ± 0.20	1.2 ± 0.02	3.30 ± 0.02	0.35 ± 0.06
CONS	20	0.994 ± 0.002	11.66 ± 0.20	2.0 ± 0.3	3.22 ± 0.02	0.40 ± 0.05

MG, Meyerozyma guilliermondii strain L11-2; CR, Candida rugosa strain UY; SC, Saccharomyces cerevisiae strain YCH497; CONS, three-species consortium. Values are mean ± SD.

3.5 Effect of fermentation temperature on wine production

Temperature interacted significantly with organism identity in shaping fermentation outcomes ($p < 0.001$; Table 3). At 10 °C, MG and CR fermented poorly (2.97 ± 0.39% and 4.33 ± 0.39% ABV, with 14.7 and 12.9 °Brix residual sugar), whereas SC and CONS remained highly effective even at this low temperature (9.58 ± 0.30% and 11.03 ± 0.39% ABV). Raising the temperature to 25 and 30 °C improved ABV in every treatment, but MG and CR continued to leave substantially more residual sugar (6.0 to 9.4 °Brix) than SC or CONS (0.7 to 1.8 °Brix) at the optimum, and produced proportionally more acetic acid as temperature rose (MG, 0.80 to 1.25 g/L; CR, 0.90 to 1.35 g/L), while SC and CONS remained at or below 0.60 g/L throughout. The consortium achieved its highest ABV (12.21 ± 0.27%) at 25 °C.

Table 3: Effect of fermentation temperature on wine production

Treatment	Temp (°C)	Final SG	ABV (%v/v)	Residual sugar (°Brix)	pH	Total acidity (g/L acetic)
MG	10	1.060 ± 0.003	2.97 ± 0.39	14.7 ± 0.9	3.52 ± 0.03	0.80 ± 0.08
MG	25	1.033 ± 0.002	6.31 ± 0.21	9.4 ± 0.7	3.48 ± 0.02	1.05 ± 0.10
MG	30	1.028 ± 0.003	7.04 ± 0.26	8.1 ± 0.6	3.47 ± 0.03	1.25 ± 0.12
CR	10	1.050 ± 0.003	4.33 ± 0.39	12.9 ± 0.8	3.48 ± 0.03	0.90 ± 0.09
CR	25	1.020 ± 0.002	8.27 ± 0.21	6.0 ± 0.6	3.40 ± 0.02	1.20 ± 0.10
CR	30	1.018 ± 0.002	8.53 ± 0.19	5.6 ± 0.5	3.38 ± 0.02	1.35 ± 0.12
SC	10	1.010 ± 0.002	9.58 ± 0.30	4.1 ± 0.3	3.40 ± 0.02	0.40 ± 0.04
SC	25	0.998 ± 0.002	11.16 ± 0.40	1.6 ± 0.2	3.28 ± 0.02	0.45 ± 0.04
SC	30	0.999 ± 0.002	11.03 ± 0.35	1.8 ± 0.2	3.27 ± 0.02	0.55 ± 0.05
CONS	10	0.999 ± 0.003	11.03 ± 0.39	1.8 ± 0.2	3.30 ± 0.03	0.35 ± 0.03
CONS	25	0.990 ± 0.002	12.21 ± 0.27	0.7 ± 0.1	3.22 ± 0.02	0.50 ± 0.05
CONS	30	0.991 ± 0.002	12.07 ± 0.30	0.9 ± 0.1	3.21 ± 0.02	0.60 ± 0.06

MG, Meyerozyma guilliermondii strain L11-2; CR, Candida rugosa strain UY; SC, Saccharomyces cerevisiae strain YCH497; CONS, three-species consortium. Values are mean ± SD.

3.6 Physicochemical and sensory quality of matured wines

After 30 days of maturation, ABV, acidity and clarity all followed the same treatment ranking established during primary fermentation, and this ranking was mirrored by the sensory panel (Table 4). ABV increased from 7.03 ± 0.06% (MG) to 12.71 ± 0.12% (CONS), residual sugar fell from 7.8 ± 0.3 to 0.06 ± 0.04 °Brix and turbidity fell from 6.2 ± 1.1 to 1.8 ± 0.4 NTU over the same range (all $p < 0.001$). Rather than trading sensory quality for fermentation efficiency, the consortium wine received the highest hedonic scores for every attribute assessed, including colour (8.4 ± 0.48), aroma (8.8 ± 0.42), taste (8.9 ± 0.35), mouthfeel (8.5 ± 0.56) and overall acceptability (8.7 ± 0.48), followed closely by SC. MG and CR, despite their slower, less complete fermentation, did not compensate with superior sensory scores; CR in particular returned the lowest ratings across all five attributes.

Table 4: Physicochemical and sensory quality of matured honey wines (30 days)

Treatment	ABV (%v/v)	Residual sugar (°Brix)	pH	Total acidity (g/L)	Turbidity (NTU)	Colour	Aroma	Taste	Mouthfeel	Overall
MG	7.03 ± 0.06	7.8 ± 0.3	3.49 ± 0.02	2.15 ± 0.08	6.2 ± 1.1	7.0 ± 0.72	6.1 ± 0.78	6.0 ± 0.75	6.5 ± 0.71	6.2 ± 0.75
CR	8.47 ± 0.08	4.5 ± 0.2	3.41 ± 0.02	2.55 ± 0.09	4.8 ± 0.9	6.5 ± 0.81	5.0 ± 0.74	4.8 ± 0.90	5.2 ± 0.61	5.1 ± 0.67
SC	11.56 ± 0.10	0.20 ± 0.08	3.30 ± 0.02	3.40 ± 0.10	2.1 ± 0.5	8.0 ± 0.63	8.2 ± 0.52	8.1 ± 0.60	7.8 ± 0.68	8.0 ± 0.63
CONS	12.71 ± 0.12	0.06 ± 0.04	3.24 ± 0.02	3.90 ± 0.12	1.8 ± 0.4	8.4 ± 0.48	8.8 ± 0.42	8.9 ± 0.35	8.5 ± 0.56	8.7 ± 0.48

MG, Meyerozyma guilliermondii strain L11-2; CR, Candida rugosa strain UY; SC, Saccharomyces cerevisiae strain YCH497; CONS, three-species consortium. Sensory attributes scored on a nine-point hedonic scale (n = 10 panellists); values are mean ± SD.

3.7 Microbiological identification and safety of matured wines

Two bacterial genera were recovered from the wines: a Gram-negative, catalase-, oxidase-, citrate- and methyl red-positive organism consistent with *Acetobacter* sp., and a Gram-positive, sugar-fermenting organism consistent with *Lactobacillus* sp. *Lactobacillus* sp. was recovered from all four treatments, while *Acetobacter* sp. was recovered only from the consortium. Total viable counts in the matured wines were low across all treatments,

ranging from 6.5×10^1 CFU/mL (CONS) to 2.8×10^2 CFU/mL (CR), and coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. were not detected in any sample (Table 5), indicating that the fermentation and maturation process yielded microbiologically safe products regardless of the starter treatment used.

Table 5: Microbiological identification and bacteriological safety of matured honey wines

Treatment	Total viable count (CFU/mL)	Coliforms	E. coli	S. aureus	Salmonella spp.	Presumptive organism(s) isolated
MG	1.0×10^2	Not detected	Absent	Absent	Absent	Lactobacillus sp.
CR	2.8×10^2	Not detected	Absent	Absent	Absent	Lactobacillus sp.
SC	1.5×10^2	Not detected	Absent	Absent	Absent	Lactobacillus sp.
CONS	6.5×10^1	Not detected	Absent	Absent	Absent	Acetobacter sp., Lactobacillus sp.

MG, Meyerozyma guilliermondii strain L11-2; CR, Candida rugosa strain UY; SC, Saccharomyces cerevisiae strain YCH497; CONS, three-species consortium.

4. DISCUSSION

4.1 Consortium synergy as the basis of superior fermentation kinetics

Every kinetic parameter measured, specific gravity decline, ABV accumulation, sugar depletion and acidification, ranked the three-species consortium above single-strain *S. cerevisiae*, which in turn outperformed the two non-*Saccharomyces* isolates. This pattern is consistent with the broader literature on mixed starter cultures, in which complementary substrate use and cross-feeding among co-fermenting species accelerate sugar consumption relative to any single strain [16],[29]. Honey must is nutritionally atypical, combining very high sugar concentration with low assimilable nitrogen [7], conditions under which single non-*Saccharomyces* strains such as *Meyerozyma guilliermondii* and *Candida rugosa* characteristically struggle to sustain glycolytic flux once ethanol and osmotic stress accumulate [30],[31]. Within the consortium, the more stress-tolerant *S. cerevisiae* component plausibly drove the bulk of sugar-to-ethanol conversion, while the two non-*Saccharomyces* partners contributed early-stage biomass and metabolic diversity before yielding to the fermentative dominance of *S. cerevisiae* as conditions became more selective, a successional pattern reported in other mixed-yeast fermentations [32].

4.2 Resolving the ethanol-aroma trade-off

The literature on non-*Saccharomyces* fermentation generally frames aroma complexity and fermentation completeness as competing outcomes: strains that enhance esters and higher alcohols tend to leave more residual sugar and lower ethanol yield [9],[10]. The present results depart from this pattern in a way that is, to our knowledge, not previously reported for mead. The consortium did not merely match *S. cerevisiae* on fermentation efficiency while sacrificing sensory quality, nor did it trade efficiency for aroma as single non-*Saccharomyces* strains did; it led on both axes simultaneously, scoring highest on every fermentation kinetic parameter and every hedonic attribute assessed by the sensory panel. This suggests that the volatile and flavour-active contributions typically attributed to non-*Saccharomyces* yeasts [33],[34] were retained within the consortium even as *S. cerevisiae* drove the fermentation to completion, rather than being lost through early competitive exclusion. If confirmed by volatile-compound profiling, this would indicate that consortium design, rather than single-strain selection, is the more promising route to mead that is both efficiently produced and sensorially distinctive.

4.3 Practical fermentation windows: must concentration and temperature

The °Brix and temperature optimisation experiments reinforced the same organism-dependent pattern and identified practical operating windows. *S. cerevisiae* and the consortium converted increasing must sugar into ethanol efficiently up to 20 °Brix with minimal residual sugar or acetic acid accumulation, whereas MG and CR left proportionally more unfermented sugar and produced more acetic acid as must strength rose, a combination associated with volatile-acidity defects in wine [35]. Temperature had an even sharper organism-dependent effect: at 10 °C, MG and CR were effectively unsuitable as sole starters, while SC and the consortium fermented efficiently even under cool conditions, a trait valuable for producers without temperature-controlled fermentation vessels. The consortium's ABV peaked at 25 °C rather than 30 °C, indicating that moderate rather than maximal fermentation temperature may best preserve the balance between ethanol yield and the acetic acid ceiling associated with sensory acceptability.

4.4 Product safety

The absence of coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. across all matured wines, combined with total viable counts below 3×10^2 CFU/mL, indicates that the low pH, ethanol content and

competitive microbial environment generated during fermentation provided an effective barrier to pathogen survival, consistent with reports linking acidification and ethanol accumulation to pathogen exclusion in fermented beverages [36]. The consistent recovery of *Lactobacillus* sp. across treatments, and additionally *Acetobacter* sp. in the consortium, is compatible with a low-level, self-limiting bacterial community that is typical of, rather than a threat to, matured fermented honey beverages [37].

4.5 Limitations and future directions

This study characterised fermentation performance and sensory acceptability at the laboratory scale using a fixed three-species consortium ratio and a single honey source, and did not include volatile-compound profiling (for example, gas chromatography-mass spectrometry) to identify the specific esters or higher alcohols underlying the sensory results, nor genomic confirmation beyond ITS sequencing. The mechanistic interpretation of consortium synergy offered here is therefore consistent with, but not yet directly demonstrated by, metabolite-level data. Future work should profile volatile aroma compounds across treatments, test alternative consortium ratios and sequential inoculation schedules, confirm strain identity with whole-genome or multilocus sequence data, and evaluate pilot-scale and shelf-life performance to establish the industrial feasibility of palm wine-derived consortia for commercial mead production.

5. CONCLUSION

Palm wine yielded three molecularly confirmed yeast species, *Saccharomyces cerevisiae* strain YCH497, *Meyerozyma guilliermondii* strain L11-2 and *Candida rugosa* strain UY, that were each capable of fermenting honey must, but a three-species consortium of these isolates consistently outperformed every single strain on fermentation kinetics, must-Brix and temperature optimisation, matured-wine physicochemistry and sensory acceptability, without compromising microbiological safety. This result indicates that the conventional trade-off between fermentation efficiency and sensory complexity in non-*Saccharomyces* fermentation can be overcome by consortium design rather than single-strain selection. Palm wine therefore represents a previously untapped, low-cost reservoir of complementary indigenous yeasts capable of converting locally abundant honey into a quality-assured, regionally distinctive mead, offering a route to value-added beverage production and rural entrepreneurship in tropical honey-producing regions.

Author Contributions

Okogu Sabinus Chukwuka: conceptualisation, investigation, formal analysis, writing – original draft. Aniaku Vincent N.: conceptualisation, methodology, supervision, writing – review and editing. Anichukwu Nwankwo Onyinye: investigation, data curation, formal analysis. Ada Ikeyi A.P.: methodology, validation, writing – review and editing.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethical Approval

This study did not involve human or animal subjects beyond a voluntary sensory panel; institutional approval details are to be inserted by the corresponding author.

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