

Fermentation Kinetics and Microbial Dynamics of Honey Mead Produced Using Palm Wine-Derived Yeast Cultures

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Abstract: Honey mead fermentation is a complex microbial process in which yeast growth, sugar utilization, ethanol production, and acidification occur simultaneously. The choice of yeast starter can substantially influence the rate and extent of these processes. This study therefore investigated the fermentation kinetics and associated microbial dynamics of honey mead produced using palm-wine-derived yeast cultures and their consortium. This study evaluated the fermentation kinetics and microbial dynamics of honey mead produced using palm-wine-derived yeast cultures. *Candida rugosa* UY, *Saccharomyces cerevisiae* YCH497, and *Meyerozyma guilliermondii* L11-2 were used individually and as an equal-proportion consortium to ferment honey must at 30°C for 14 days. Fermentation progress was monitored through specific gravity, alcohol production, apparent soluble solids, pH, total acidity, and viable yeast populations. The consortium demonstrated the highest fermentation efficiency, with specific gravity decreasing from 1.083 to 0.989 and apparent soluble solids declining to 0.25 °Brix by day 14. It also produced the highest alcohol content (12.22%), compared with 11.16%, 8.27%, and 6.82% for *S. cerevisiae*, *C. rugosa*, and *M. guilliermondii*, respectively. The consortium showed the greatest acidification, with final pH and total acidity of 3.22 and 4.01 g/L, respectively. Yeast populations increased during early fermentation, reaching their highest levels around day 4, followed by a decline toward day 14. Presumptive *Acetobacter* and *Lactobacillus* isolates were associated with the fermented mead. Overall, the findings demonstrate that the yeast consortium provided superior and more coordinated fermentation performance compared with the individual cultures, highlighting its potential for efficient honey-mead production.

Keywords: Honey mead; Fermentation kinetics; Microbial dynamics; Palm wine-derived yeasts; Yeast consortium; *Saccharomyces cerevisiae*; *Candida rugosa*; *Meyerozyma guilliermondii*; Ethanol production; Sugar utilization.

1. INTRODUCTION

Mead is an alcoholic beverage produced principally through the fermentation of diluted honey by yeasts and has received renewed scientific and commercial interest because of its distinctive physicochemical and sensory characteristics. The fermentation process is strongly influenced by honey composition, yeast strain, nutrient availability, temperature, pH and initial sugar concentration, making starter-culture selection a critical determinant of fermentation performance and final product quality (Jose-Salazar *et al.*, 2024).

Honey must presents several physiological challenges to fermenting yeasts, including high osmotic pressure, an acidic environment and relatively low concentrations of yeast-assimilable nitrogen. Consequently, sluggish or incomplete fermentation may occur unless appropriate yeast strains and nutritional conditions are employed. *Saccharomyces cerevisiae* remains the predominant starter organism because of its robust fermentative capacity and tolerance to ethanol and acidic conditions; however, considerable strain-dependent differences in fermentation performance have been reported (Webster *et al.*, 2025). Increasing attention has therefore been directed toward non-*Saccharomyces* yeasts and mixed-culture fermentation. Non-*Saccharomyces* species can modify fermentation behaviour and contribute metabolic diversity and desirable sensory characteristics, although their growth and ethanol-producing capacities may differ from those of *S.*

cerevisiae. Co-inoculation can consequently provide an alternative strategy for combining the fermentative robustness of *Saccharomyces* with the metabolic contributions of other yeasts (Jose-Salazar *et al.*, 2024).

Fermentation kinetics provide a more informative assessment of starter performance than final ethanol concentration alone. Monitoring changes in sugar concentration, specific gravity, ethanol production, pH, acidity and microbial population over time allows the progression of substrate utilization, yeast growth and product formation to be quantitatively evaluated. Recent studies have demonstrated the usefulness of kinetic approaches and mathematical models for describing yeast growth, substrate consumption and ethanol production during mead fermentation (Fentie *et al.*, 2026).

Palm wine represents an important indigenous reservoir of fermentation microorganisms in Nigeria. The spontaneous fermentation of palm sap involves diverse yeasts, including *S. cerevisiae* and several non-*Saccharomyces* species, and previous studies have demonstrated the technological potential of yeasts isolated from Nigerian palm wine for alcoholic fermentation (Osiebe *et al.*, 2023; Oladoja *et al.*, 2021). Despite this microbial potential, comparatively limited attention has been given to the temporal fermentation behaviour of palm wine-derived yeasts in honey must. In particular, the relationships among yeast population development, sugar depletion, ethanol accumulation and acidification remain insufficiently characterized. Therefore, this study aims to investigate the fermentation kinetics and microbial dynamics of honey mead produced using palm wine-derived yeast cultures, with particular emphasis on changes in specific gravity, alcohol production, residual sugar, pH, total acidity and viable yeast populations during fermentation.

2. MATERIALS AND METHODS

2.1 Source of Yeast Cultures

Palm wine samples were obtained from *Elaeis guineensis* (oil palm) and *Raphia hookeri* (raffia palm) trees in Ugwuomu-Nike, Enugu State, Nigeria. Fresh sap was collected aseptically between 06:00 and 08:00 h directly from the tapping receptacles into sterile 1-L amber bottles. Samples were transported to the laboratory on ice at approximately 4°C and processed within 12 h of collection. Three yeast isolates previously recovered from the palm wine samples were used as fermentation starters: *Candida rugosa* UY, *Saccharomyces cerevisiae* YCH497, and *Meyerozyma guilliermondii* L11-2. The isolates were characterized morphologically and identified by ITS-region sequencing and BLAST comparison against GenBank reference sequences. *C. rugosa* UY, *S. cerevisiae* YCH497, and *M. guilliermondii* L11-2 showed 98.55%, 92.14%, and 99.11% sequence similarity, respectively, to the corresponding reference sequences, as reported by Okogu *et al.* (2026).

2.2 Preparation and Standardization of Yeast Inocula

Each yeast isolate namely *Saccharomyces cerevisiae* YCH497, *Meyerozyma guilliermondii* L11-2, and *Candida rugosa* UY was activated separately by inoculating a loopful of stock culture into Yeast Extract Peptone Dextrose (YEPD) broth and incubating at $28 \pm 2^\circ\text{C}$ for 48 h under stationary conditions to obtain actively growing cultures. Cell density of each activated culture was standardized spectrophotometrically to an optical density (OD₆₀₀) of 0.5 at a wavelength of 600 nm, a standard wavelength for estimating microbial cell concentration in liquid culture, ensuring uniform starting cell loads across isolates prior to fermentation. Viable cell counts corresponding to the standardized OD were confirmed by serial dilution and spread plating on YEPD agar, followed by incubation at 28°C for 48 h and colony enumeration (expressed as colony-forming units per millilitre, CFU/mL).

For fermentation studies, yeast biomass was propagated under the same standardized conditions in shake flasks, harvested by centrifugation at 125 rpm for 15min, and the resulting cell pellet was resuspended in sterile distilled water (or saline) to serve as the working inoculum. Each honey must was inoculated at a rate of 5 g/L (wet biomass), equivalent to a 0.5% (w/v) inoculum concentration, to standardize the initial cell loading across all fermentation treatments.

Four fermentation treatments were established based on inoculum composition:

- SC — *Saccharomyces cerevisiae* YCH497 (monoculture)
- MG — *Meyerozyma guilliermondii* L11-2 (monoculture)
- CR — *Candida rugosa* UY (monoculture)
- CONS — a mixed-culture consortium of SC, MG, and CR combined in equal proportions

For the consortium (CONS), equal volumes of the three standardized cell suspensions were combined to achieve equal proportional representation of each species, while the total inoculum concentration was maintained at 0.5% (w/v), identical to the monoculture treatments, to ensure that any observed fermentation differences reflected microbial interactions rather than differences in total inoculum load.

2.3 Preparation of Honey Must

Raw, unadulterated honey was obtained from certified beekeepers in Opi, Nsukka, Enugu State, Nigeria, and stored in sterile airtight food-grade containers at 25 °C in darkness until processing. Honey must was prepared aseptically by dilution of the honey to an initial concentration of approximately 20 °Brix for the primary kinetic experiment. The must pH was adjusted to 3.5 using food-grade citric acid. Diammonium phosphate was incorporated at 0.5 g/L as a nitrogen supplement. The prepared honey must was pasteurized at 70 °C for 1 min and allowed to cool under aseptic conditions before inoculation.

2.4 Preparation of Yeast Inoculum

Pure yeast isolates were activated by subculturing into sterile yeast extract–peptone–dextrose (YEPD) broth and incubating at 28 °C for 48 h. Following activation, the cultures were standardized to an optical density (OD) of 0.5 to ensure uniform inoculation. Viable cell concentrations were determined by serial dilution and spread plating on YEPD agar, followed by incubation at 28 °C for 48 h. The resulting colonies were counted and expressed as colony-forming units per millilitre (CFU/mL).

2.5 Fermentation of Honey Must

The standardized yeast preparations were used to inoculate the honey must treatments. The dried yeast biomass, at 5 g, was added to 1000 mL of sterile honey must containing 5 g of yeast extract. Fermentation was conducted in 1.5-L glass fermenters at 30°C for 14 days as the primary fermentation period. The four fermentation treatments comprised individual fermentations with *C. rugosa* UY (CR), *S. cerevisiae* YCH497 (SC), *M. guilliermondii* L11-2 (MG), and an equal-proportion consortium of the three yeast isolates (CONS). Fermentation samples were collected at the designated sampling intervals for kinetic and microbiological analyses.

2.6 Determination of Specific Gravity and Sugar Content

The progress of fermentation was monitored by determining the specific gravity (SG) of the wine samples at 48-hour intervals following the methods described by Pires *et al.* (2014) and Anyanwu and Obi (2022). A measured volume of the fermenting wine was transferred into a clean and dry measuring cylinder. A calibrated hydrometer was carefully lowered into the sample and allowed to float freely without touching the sides of the cylinder. The specific gravity reading was taken at eye level from the meniscus and recorded. Measurements were carried out throughout the fermentation period. Changes in specific gravity were used to estimate sugar depletion and monitor ethanol production, with decreasing SG values indicating progressive conversion of sugars into alcohol by fermenting microorganisms.

2.7 Determination of Alcohol Content

Alcohol content was determined using an alcohol meter. Fermentation samples were first filtered to remove suspended materials and transferred into a clean measuring cylinder. The alcohol meter was immersed carefully and allowed to float freely without touching the cylinder walls. After stabilization, the alcohol percentage was read directly from the calibrated scale at eye level and recorded.

2.8 Determination of Residual Sugar

Residual sugar content of the wine samples was determined using a Brix refractometer according to the method described by Aniaku and Ogunbode (2022). The refractometer prism was cleaned and calibrated with distilled water before use. A few drops of the wine sample were placed on the prism surface and the cover plate was closed gently. The refractometer was directed toward a light source, and the °Brix value was read directly from the scale. The reading obtained was recorded as the residual sugar content of the wine sample.

2.9 Determination of pH

The pH of the fermenting mead was determined using a calibrated digital pH meter. A 10-mL portion of each sample was transferred into a clean beaker, and the electrode of the pH meter was immersed in the sample. The pH value was recorded after the reading had stabilized.

2.10 Determination of Total Acidity

Ten (10 g) of honey sample was dissolved in 75 ml of carbon dioxide-free distilled water. The solution was stirred and the pH meter was calibrated with a standard buffer solution before immersing the electrodes into the mixture to obtain the initial pH. The solution was then titrated with 0.1 M NaOH until the pH reached 8.30, indicated by a stable reading or faint pink colouration when phenolphthalein was used as an indicator. The volume of NaOH used was recorded. Free acidity was calculated using the formula:

$$\text{Free acidity (meq/kg)} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 1000}{\text{Mass of honey (g)}}$$

2.11 Enumeration of Viable Yeast Population

Yeast population dynamics during primary fermentation were determined using the standard plate-count technique. Samples were collected aseptically on days 0, 2, 4, 7, and 14. One millilitre of fermenting must was transferred into 9 mL of sterile physiological saline to obtain a 10^{-1} dilution. Serial tenfold dilutions were subsequently prepared to 10^{-6} . Aliquots of 0.1 mL from appropriate dilutions were spread-plated in triplicate onto yeast-peptone-dextrose (YPD) agar. Plates were incubated at 30°C for 48 h. Colonies were counted and the viable yeast population was expressed as CFU/mL and subsequently transformed to $\log_{10}$ CFU/mL for statistical analysis.

2.12 Isolation of Associated Bacteria

Bacterial populations associated with the produced mead were assessed by serial dilution and culture-based isolation. One millilitre of each mead sample was transferred into 9 mL of sterile normal saline, followed by serial tenfold dilution to 10^{-6} . Aliquots of 0.1 mL from appropriate dilutions were inoculated onto Nutrient Agar, MacConkey Agar, and Salmonella-Shigella Agar. The plates were incubated at 37°C for 48 h. Colonies were enumerated and representative isolates were purified for subsequent characterization. Platings were performed in duplicate Cheesbrough, (2006).

2.13 Morphological and Biochemical Characterization of Bacterial Isolates

Bacterial isolates were characterized based on colony morphology, Gram-staining reaction, and biochemical characteristics. Gram staining was performed using crystal violet, Gram's iodine, alcohol decolorization, and safranin counterstaining, followed by microscopic examination under oil immersion. The isolates were subjected to catalase, coagulase, citrate utilization, indole, oxidase, and methyl-red tests and Sugar fermentation using standard procedures according to the method of Cheesbrough, (2006).

2.14 Statistical Analysis

Experimental results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using SPSS version 25 and/or GraphPad Prism 9. Differences among fermentation treatments and sampling periods were evaluated using analysis of variance, with statistical significance established at $p < 0.05$. Where significant differences occurred, appropriate post-hoc mean-separation procedures, including Tukey's HSD or Duncan's Multiple Range Test where applicable, were used.

3. RESULTS

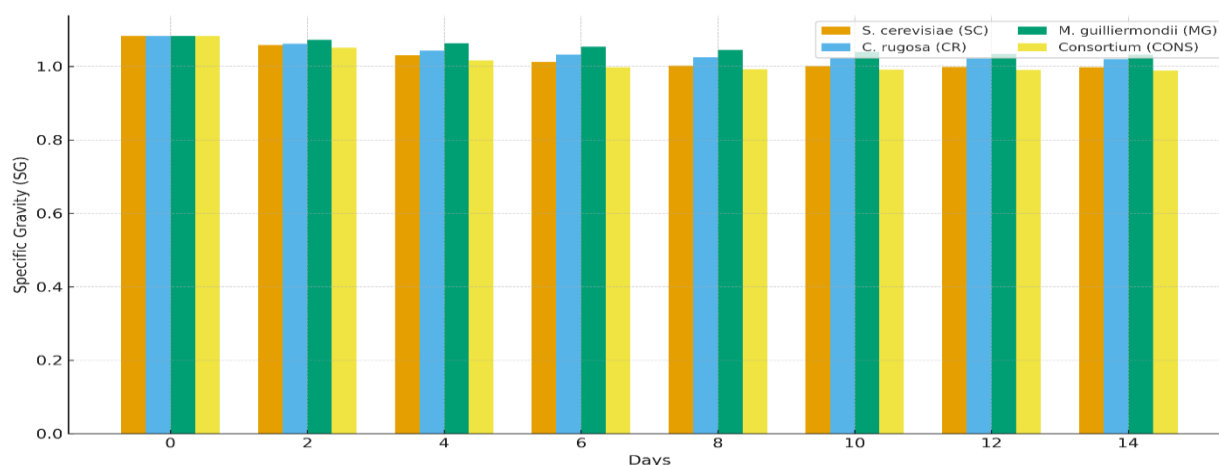


Figure 3.1: Specific Gravity (SG) Changes during Primary Fermentation of Honey

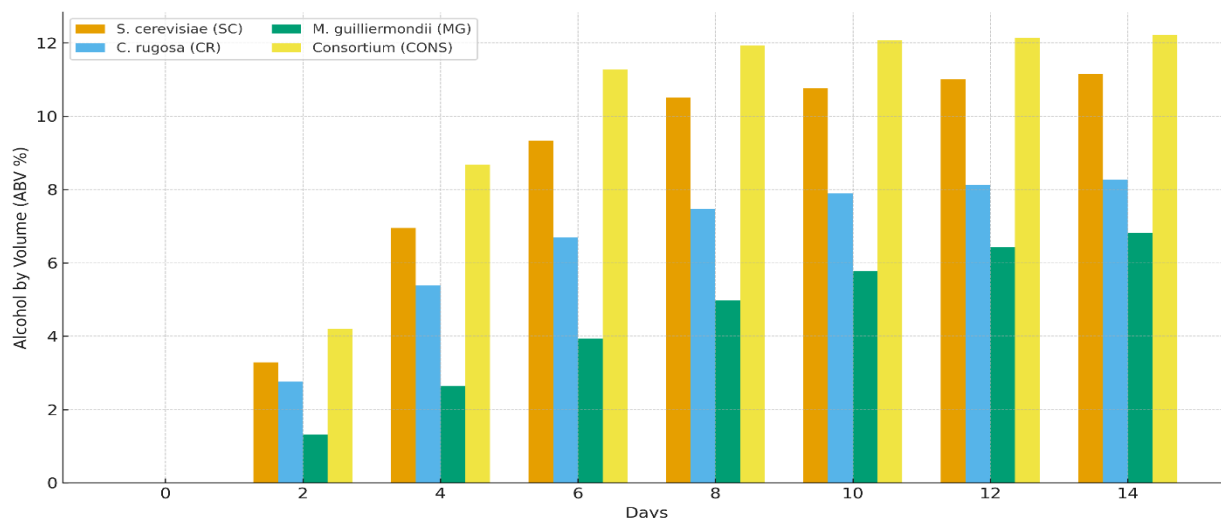


Figure 3.2: Alcohol Changes during Primary Fermentation of Honey

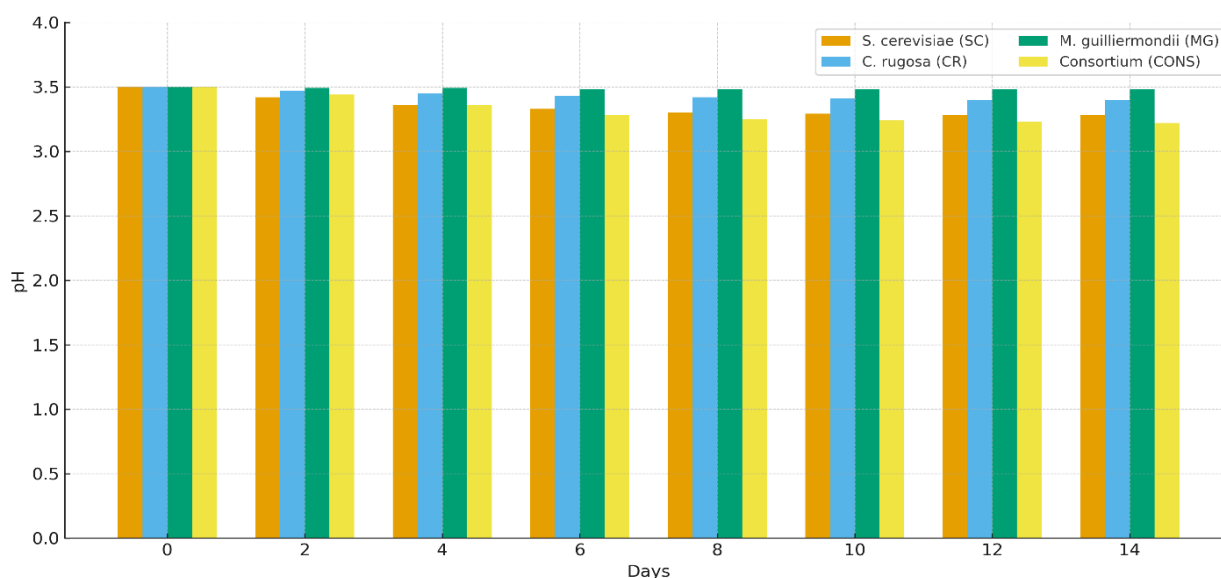


Figure 3.3: pH Changes during Primary Fermentation of Honey

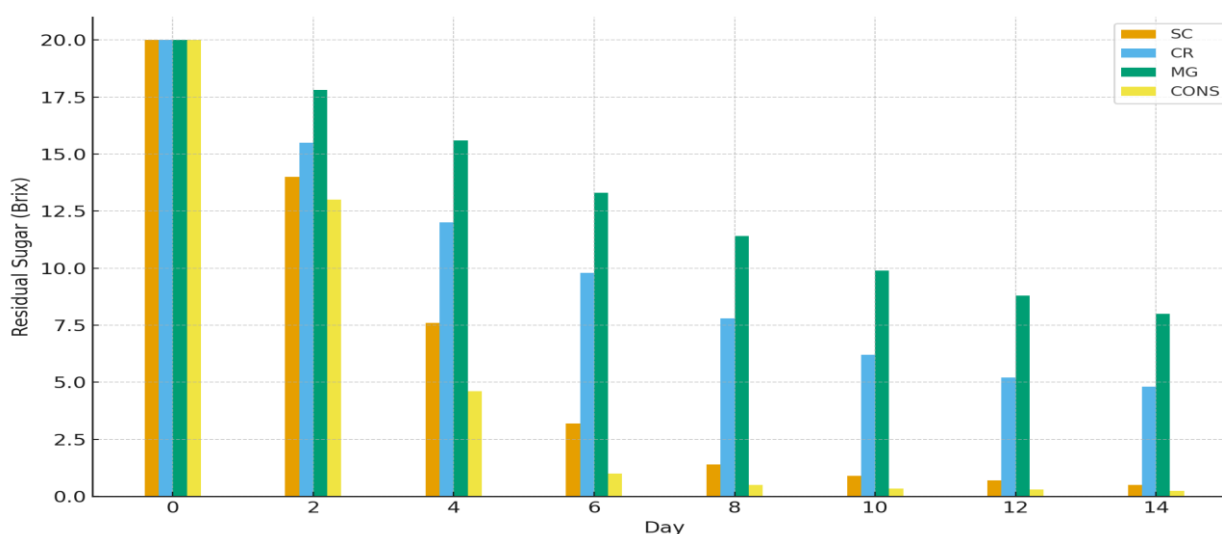


Figure 3.4: Residual Sugar (Brix) profile during Primary Fermentation of Honey

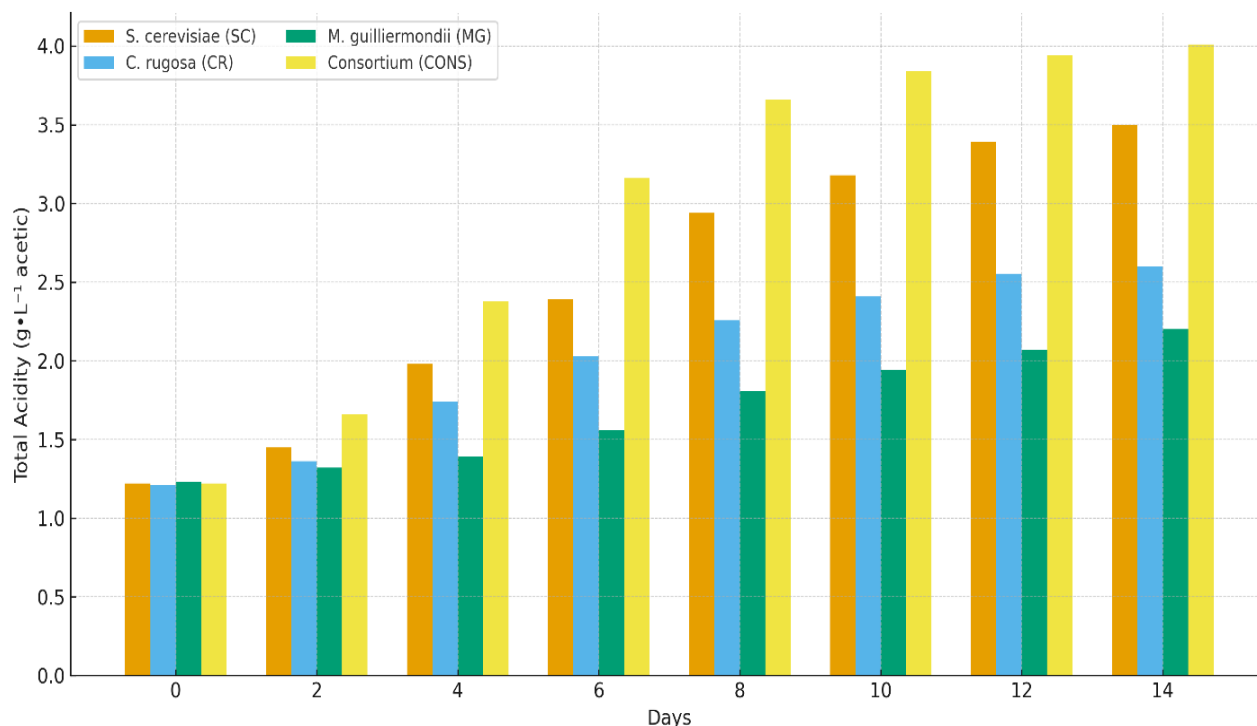


Figure 3.5: Total Acidity Changes during Primary Fermentation of Honey

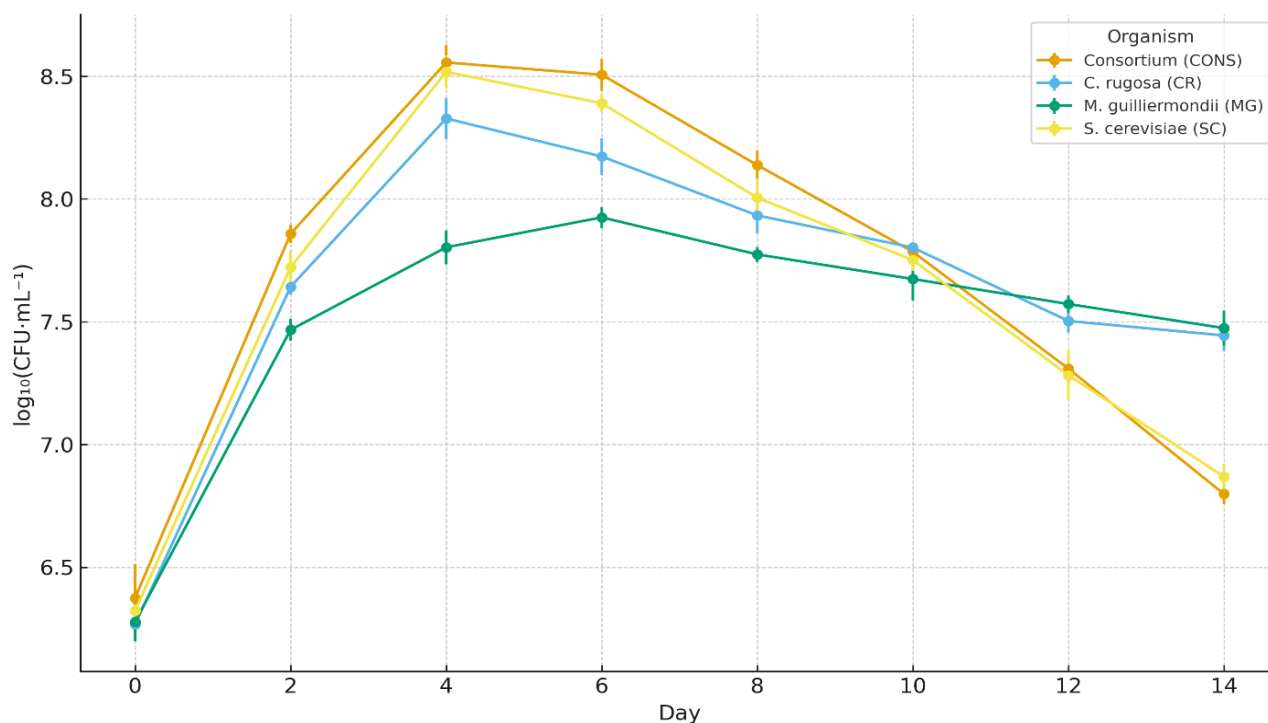


Figure 3.6: Yeast Growth During Primary Fermentation of Honey

Table 3.1: Identification of Bacterial Isolates from Honey Must Wine Samples

S/N	Growth Appearance on Nutrient Agar	Gram Reaction	Catalase Test	Oxidase Test	Citrate Test	Coagulase Test	Methyl Red Test	Indole Test	Glucose	Fructose	Maltose	Mannitol	Lactose	Suspected Organisms
1	Creamy colonies, irregular edges on Nutrient Agar	Gram –ve Bacteria	+	+	+	–	+	–	A	A	–	–	–	<i>Acetobacter</i> sp
2	Small white colonies, smooth surface on Nutrient Agar	Gram +ve Bacteria	–	–	–	–	+	–	A	A	AG	A	A	<i>Lactobacillus</i> sp.

KEY: + = positive, - = negative, A = Acidic, AG = Acidic and Gas, G = Gas, +ve = positive, —ve = negative

4. DISCUSSION

4.1 Overview of Fermentation Dynamics

The fermentation profiles demonstrate that honey-mead fermentation was a dynamic process involving closely related changes in yeast growth, substrate utilization, ethanol accumulation and acidification. Although all treatments began under comparable conditions, clear differences emerged during the early stages of fermentation. This pattern is consistent with recent mead studies in which temporal measurements of microbial growth, sugar consumption, ethanol production and pH were used collectively to characterize fermentation kinetics (Jose-Salazar *et al.*, 2024). The early divergence among genetically distinct inocula, despite identical must composition, indicates that starter identity rather than substrate availability was the primary driver of fermentation trajectory in this system, supporting inoculum selection as the more decisive practical control point for mead producers.

4.2 Specific Gravity, Sugar Utilization and Ethanol Accumulation

During the initial phase, rapid yeast proliferation was accompanied by progressive substrate utilization. The similarity in initial specific gravity among treatments (approximately 1.083; $p = 0.443$) indicates comparable starting conditions, whereas significant divergence occurred from day 2 onward. CONS exhibited the greatest reduction in specific gravity, reaching 0.989 by day 14, while MG retained the highest value at approximately 1.031 (Figure 3.1). The decline in SG corresponded closely with the reduction in residual sugar (Figure 3.4) and the increase in ethanol concentration (Figure 3.2), demonstrating that the decrease in density was associated with progressive conversion of fermentable substrates into fermentation products. This integrated pattern is consistent with kinetic studies showing that substrate depletion and ethanol formation provide complementary indicators of fermentation progress (Jose-Salazar *et al.*, 2024).

The consortium displayed the most rapid and extensive substrate conversion. Residual sugar declined from approximately 20 °Brix initially to 4.60 °Brix by day 4 and 0.25 °Brix by day 14, whereas SC reached 0.50 °Brix and CR and MG retained considerably more sugar (Figure 3.4). This was accompanied by the highest ethanol accumulation in CONS, which reached 4.20% ABV by day 2 and 12.22% by day 14, compared with 11.16%, 8.27% and 6.82% for SC, CR and MG, respectively (Figure 3.2). Thus, the combined SG, residual-sugar and ethanol profiles provide convergent evidence that overall fermentation performance followed the order CONS > SC > CR > MG. This approach treating microbial growth, substrate consumption and product formation as interconnected kinetic variables rather than isolated endpoints mirrors recent kinetic

modelling of a mixed-culture honey-wine system co-inoculated with *Saccharomyces cerevisiae* and a bacterial partner, in which time-series growth, substrate depletion and product accumulation were fitted as a single interacting kinetic system (Fentie *et al.*, 2026).

CONS's near-total sugar exhaustion (0.25 °Brix) and highest ethanol yield (12.22%) is consistent with the general finding, across mixed-inoculum fermentation studies, that multi-organism starters more completely convert substrate than any single constituent strain (Fentie *et al.*, 2026). MG's comparatively high residual sugar (retaining considerably more sugar than the other treatments) and lowest ethanol yield (6.82%) closely mirrors the species-level pattern reported for *Meyerozyma guilliermondii*, which showed a lower fermentative capacity and a lower conversion rate of sugar to alcohol relative to *S. cerevisiae* in a comparative fermentation trial (Zhao *et al.*, 2022). The near-complete sugar depletion and highest ABV in CONS suggest this consortium could shorten fermentation time and lower the risk of spoilage from residual fermentable sugar a practically useful outcome for scaled mead production, provided sensory quality is not compromised.

4.3 Comparative Performance of the Yeast Cultures

The strong performance of CONS may reflect the combined physiological characteristics of the constituent yeasts, although the present data do not establish a specific mechanism such as metabolic cross-feeding. The particularly strong performance of SC is consistent with its established robustness in alcoholic fermentation, whereas the slower sugar utilization and ethanol production of CR and MG indicate lower fermentative performance under the conditions used. For MG specifically, this pattern directly parallels findings from a comparative fermentation trial in which *Meyerozyma guilliermondii* showed a lower fermentative capacity and lower sugar-to-alcohol conversion than *S. cerevisiae*, alongside a higher yield of volatile acidity (Zhao *et al.*, 2022) a trade-off consistent with MG's slower ethanol accumulation but non-trivial acid contribution in the present study (Section 4.4).

For CR, the comparison is less straightforward. Elsewhere in the literature, *Candida rugosa* (frequently reclassified in recent taxonomy as *Diutina rugosa*) has shown ethanol yields and productivities comparable to engineered *S. cerevisiae* strains under glucose/xylose co-fermentation conditions (Batista *et al.*, 2025) considerably stronger performance than CR displayed here. This divergence suggests that *C. rugosa*'s fermentative capacity is strongly substrate- and strain-dependent rather than a fixed species trait, and that the isolate used in this study (UY) may simply be a weaker fermenter than the strain characterized by Batista *et al.* (2025), or may be better suited to different sugar profiles than the honey-based must used here.

Importantly, the slower fermentation of MG and CR should not automatically be interpreted as technological inferiority, since non-*Saccharomyces* yeasts may contribute characteristics other than rapid ethanol production. Yeast selection and fermentation conditions have been shown to substantially influence both fermentation kinetics and final beverage characteristics more broadly in mead systems (Webster *et al.*, 2025).

The ranking observed here (CONS > SC > CR > MG) is broadly consistent with the wider pattern in mixed-species mead and wine literature, where *Saccharomyces*-based or mixed inocula tend to outperform single non-*Saccharomyces* strains on fermentation rate and ethanol yield (Zhao *et al.*, 2022; Webster *et al.*, 2025), even though non-*Saccharomyces* species are often retained deliberately for aromatic or acid contributions rather than speed. Given that CR's fermentative weakness may be strain-specific rather than species-typical (Batista *et al.*, 2025), a practical next step would be to test whether *C. rugosa* UY's performance improves under different sugar concentrations or supplementary nitrogen, before concluding it is unsuitable for honey-must fermentation generally. MG and CR may be better positioned as blending partners for aroma or acid contribution alongside CONS or SC, rather than as standalone fermentation drivers.

4.4 Acidification Trends (pH and Total Acidity)

The physicochemical changes were accompanied by progressive acidification. The pH of all treatments began at approximately 3.50 and subsequently declined, with CONS reaching the lowest final value (3.22), followed by SC (3.28), CR (3.40) and MG (3.48) (Figure 3.3). At the same time, total acidity increased throughout fermentation, reaching approximately 4.01 g/L in CONS compared with 3.50, 2.60 and 2.20 g/L in SC, CR and MG, respectively (Figure 3.5). The inverse relationship between pH and total acidity demonstrates progressive acidification of the fermenting must. The particularly strong acidification in CONS occurred alongside its greater sugar utilization and ethanol production, indicating that the consortium generated the most pronounced overall metabolic transformation of the honey must. The lower terminal

pH in CONS (3.22) may confer a microbial-stability advantage in the finished product, since lower pH generally suppresses spoilage organisms more effectively than the higher pH retained by CR and MG linking the kinetics findings directly to a shelf-stability outcome relevant to quality control.

4.5 Yeast Growth Kinetics: Early Proliferation

The temporal yeast-growth profile provides further insight into this process. CONS and SC showed the strongest early population increases, reaching 8.556 ± 0.073 and $8.518 \pm 0.067 \log_{10}$ CFU/mL, respectively, by day 4 (Figure 3.6). This period coincided with rapid sugar depletion and ethanol accumulation (Figures 3.2, 3.4 and 3.6), suggesting that the early growth phase represented the period of most intensive fermentation activity. This relationship is consistent with kinetic modelling of mixed-culture fermentation, in which biomass growth, substrate depletion and product formation were shown to follow closely related but temporally distinct trajectories, with growth and substrate consumption best captured by logistic-type models and product formation better described by Monod-type models (Fentie *et al.*, 2026). The near-identical peak population densities of CONS and SC (8.556 vs $8.518 \log_{10}$ CFU/mL) indicate that the consortium's overall advantage (Section 4.2) did not arise from a larger absolute biomass, but from how that biomass converted substrate consistent with the view that growth and product-formation kinetics are governed by partly independent mechanisms even when strongly correlated in time (Fentie *et al.*, 2026).

4.6 Yeast Growth Kinetics: Late-Stage Decline

An important feature of the results was the subsequent decline in viable yeast populations. By day 14, CONS and SC had declined to approximately 6.80 and 6.87 $\log_{10}$ CFU/mL, respectively, whereas CR and MG retained higher populations (Figure 3.6). This apparent reversal demonstrates that late-stage viable-cell abundance did not directly correspond to overall fermentation efficiency. CONS had the lowest late viable population but simultaneously exhibited the lowest residual sugar, lowest SG and highest ethanol concentration (Figures 3.1, 3.2, 3.4 and 3.6). The decline in culturable cells may therefore reflect increasing physiological stress associated with ethanol accumulation, acidification and depletion of readily available substrates rather than cessation of fermentation. This interpretation is consistent with mechanistic work on *Saccharomyces cerevisiae* during wine alcoholic fermentation, where yeast cell death has been linked to ethanol-induced membrane stress and micronutrient/nitrogen limitation, occurring independently of whether fermentation itself has stopped (Duc *et al.*, 2017).

This decoupling of viable-cell counts from fermentative efficiency carries a methodological implication for future monitoring: viable plate counts alone would understate the fermentative achievement of CONS and SC if used as the sole progress indicator. Multi-parameter monitoring (SG, sugar, ethanol, pH, alongside cell counts) is needed to avoid mistaking late-stage population decline for fermentation failure.

4.7 Bacterial Community Involvement

The bacterial findings provide an additional dimension to this fermentation trajectory. Presumptive *Lactobacillus* sp. was recovered from the fermented treatments, while *Acetobacter* sp. and *Lactobacillus* sp. were recovered from the consortium. The occurrence of these bacteria is relevant because lactic acid bacteria can participate in acid-related transformations, while *Acetobacter* possesses oxidative metabolism capable of converting ethanol to acetic acid under suitable oxygen conditions. A comparable pattern has been reported in mead fermented with a combined *Saccharomyces cerevisiae*–lactic acid bacteria inoculum, where mixed yeast–bacteria fermentation altered the physicochemical trajectory of the mead including ethanol yield relative to yeast-only fermentation (Fu *et al.*, 2023). The recovery of *Acetobacter* specifically from CONS is noteworthy because this treatment also displayed the highest total acidity and lowest pH (Figures 3.3 and 3.5). However, the association does not demonstrate that *Acetobacter* caused the greater acidity, because individual organic acids and bacterial population dynamics were not quantified. The bacterial results are therefore better interpreted as evidence of an associated microbial community that may have contributed to secondary biochemical transformations.

The co-occurrence of lactic acid bacteria with yeast populations, and its association with altered physicochemical outcomes, aligns with the Fu *et al.* (2023) finding that adding a bacterial partner to yeast-driven mead fermentation measurably shifts the beverage's composition though that study did not isolate *Acetobacter* specifically, so the acetic-acid-bacteria dimension of the present findings remains without a directly matching mead-specific precedent. The presence of *Acetobacter* alongside the highest-acidity treatment is a caution flag for process control: uncontrolled, acetic acid bacteria could push CONS

toward excessive volatile acidity in longer fermentations or under more aerobic conditions, so headspace oxygen management may be a relevant control point for any future scale-up of this consortium.

4.8 Integrated Fermentation Sequence

Overall, the six fermentation profiles describe a coherent sequence: rapid early yeast growth (Fig. 3.6) → accelerated sugar depletion (Fig. 3.4) → declining specific gravity (Fig. 3.1) → increasing ethanol (Fig. 3.2) → increasing acidity and declining pH (Figs. 3.3, 3.5) → late-stage decline in viable yeast populations (Fig. 3.6). The consortium showed the strongest expression of this sequence, demonstrating the fastest and most extensive fermentation among the treatments. This integrated interpretation is consistent with kinetic modelling of mixed-culture honey-wine fermentation, where time-series microbial growth, substrate consumption and product formation were treated as interconnected components of a single kinetic system (Fentie *et al.*, 2026). Because CONS traversed this sequence fastest and most completely, it represents the most time-efficient of the four treatments for practical mead production, provided downstream sensory attributes (aroma, mouthfeel, volatile-acidity load) prove acceptable a dimension not assessed in the present kinetic dataset.

4.9 Principal Finding and Limitations

Thus, the principal finding is not simply that CONS produced the highest final alcohol concentration. Rather, the superiority of the consortium is demonstrated by the convergence of multiple independent kinetic indicators SG reduction, sugar depletion, ethanol accumulation, acidification and early yeast proliferation. This provides stronger evidence for enhanced collective fermentation performance than reliance on any single endpoint. Nevertheless, because species-specific dynamics within the consortium and bacterial metabolite production were not directly measured, the physiological mechanisms underlying its superior performance remain to be established through future molecular and metabolite-level investigations. Taken together, the findings support consortium-based inoculation (SC + MG + CR) as a more robust strategy than single-strain fermentation for honey-mead production under the conditions tested, while identifying species-specific gaps particularly the strain-dependence of *C. rugosa*'s fermentative capacity (Section 4.3) and the unquantified contribution of *Acetobacter* to acidification (Section 4.7) as priorities for follow-up molecular and metabolite-level work.

5. CONCLUSION

The study demonstrated that fermentation kinetics and microbial dynamics of honey mead were strongly influenced by yeast composition. The consortium showed the best overall performance, with the greatest sugar utilization, specific gravity reduction, alcohol production, and acidification. Yeast populations increased during early fermentation before declining toward the end of primary fermentation, while presumptive *Acetobacter* and *Lactobacillus* isolates were associated with the fermented mead. These findings have practical relevance to controlled wine and fermented-beverage production, particularly in the selection of mixed starter cultures and monitoring of fermentation efficiency and microbial stability. Overall, the palm-wine-derived yeast consortium demonstrated strong potential for efficient honey-mead fermentation and warrants further evaluation at pilot and industrial scales.

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