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Influence of yeast application and fermentation duration on the physicochemical properties and bacterial community of *Coffea arabica* var. Bourbon

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Abstract

This study investigates the effects of yeast application with fermentation durations on physicochemical properties such as total soluble solids (TSS), titratable acidity (TA), and potential hydrogen (pH), and microbial colony-forming units (CFU/g) of bacteria in Arabica coffee fermentation. Yeast and fermentation duration produced a substantial combined effect on the properties of coffee. The sugar metabolism activities in yeast-fermented coffee reduced TSS, although TA levels increased and pH measurement decreased because of acid accumulation. The colony-forming unit (CFU/g) of bacteria varied significantly in all treatments, with yeast treatments increasing CFU/g after fermentation and gradual reduction after drying, while longer fermentation durations influenced the decrease of CFU/g but led to increased CFU/g after drying, particularly at 72 hours. The statistical analysis revealed highly significant results across all treatment combinations. This is how yeast fermentation under controlled conditions shapes sugar metabolism during the fermentation process, as well as affects acid production and microbial populations, which results in enhanced coffee quality properties.

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Introduction

Post-harvest fermentation of coffee is essential in shaping the sensory characteristics and the quality of coffee products. The biochemical transformation of coffee mucilage is conducted by microbes during fermentation, resulting in changes in the physicochemical properties and microbial composition of coffee beans (Silva *et al.*, 2013). Scientists extensively study *Saccharomyces cerevisiae* yeast strains because they enhance coffee quality by controlling acidity and flavor development and creating desirable volatile compounds (Wang *et al.*, 2020).

Coffee attributes in the final product depend heavily on the fermentation time because this phase determines how sugars break down while influencing organic acid formation and microbial population shifts (Silva *et al.*, 2019).

Recent studies highlight how controlled fermentations require yeast inoculation because this method improves coffee characteristics. According to Zhang *et al.* (2020), yeast fermentation produces substantial changes in coffee acidity and pH that influence how people perceive sensory qualities. The fermentation process enables microbial interactions to regulate bacterial populations that destroy mucilage while creating unique flavor compounds (Vilela *et al.*, 2010).

A study conducted by Wang *et al.* (2020) showed that specific yeast strains application for fermentations could provide distinct coffee flavor sensations while at the same time improving product stability.

Few studies have explored how yeast application interacts with fermentation duration when processing *Coffea arabica* var. Bourbon in the specific environmental conditions found in Central Mindanao, Philippines. Research about yeast inoculation combined with fermentation time implications on physicochemical characteristics and bacterial communities of *Coffea arabic*a var. Bourbon is vital for maximizing processing optimization and improving coffee quality. This research investigates controlled yeast fermentation effects on the physicochemical properties and microbial changes of *Coffea arabica* var. Bourbon is grown in Central Mindanao, Philippines.

Materials and methods

Experimental area

The fermentation setup was conducted at Banisilan, North Cotabato, Philippines. A total of 54 kilograms of de-pulped Arabica coffee cherries were used for the fermentation process, 27 kilograms were allotted to be treated with yeast, and 27 kilograms were not treated with yeast, with 3 kilograms allocated for each treatment combination. According to treatment combinations, a 300-gram sample of fermented and dried coffee beans was subjected to microbial count at the Microbiology Research Laboratory, College of Veterinary Medicine, Central Mindanao University. Furthermore, a sample was sent to the Department of Science and Technology (DOST) - XI, Regional Standards and Testing Laboratories in Davao City for the physicochemical property analysis of fermented and dried coffee beans. The fermentation process used polyethylene UV plastic (200 microns) for fermentation, a pH meter, refractometer, storage container, and hygrometer are used for the fermentation process. For microbial populations, Petri dishes, agar media, inoculation loops, incubators, microscopes, microscope slides, coverslips, colony counters, Gram stain kits, biochemical test kits, and sterilization equipment.

Sources and selection of coffee cherries

Coffee beans (*Coffea arabica* var. Bourbon) were harvested from Sitio Balutakay, Managa, Bansalan, Davao del Sur, Philippines, which is located at an elevation of approximately 1,268.3 meters (4,161 feet) above sea level (PhilAtlas, 2024). Elevation plays a critical role in coffee quality, influencing bean density, acidity, and overall flavor profile (Sweet Maria's Coffee Library, 2018). The Arabica coffee generally starts bearing fruit within two to four years after planting, with cherries reaching full maturity seven to eight months after flowering (Eerie Coffee Company,

2024). The maturation process consists of an early development phase lasting about three to four months, followed by a ripening phase of approximately four months, during which the cherries change from green to deep red (Sweet Maria's Coffee Library, 2018). To ensure high-quality coffee, cherries were selected based on four key criteria: (1) Ripeness, where only fully ripe, red cherries were harvested to enhance flavor and sugar content; (2) Health, ensuring that cherries were free from diseases and physical damage to prevent processing defects; (3) Uniformity, selecting cherries of consistent size and density to promote even roasting and flavor consistency; and (4) Harvesting Method, employing selective picking, in which skilled workers carefully handpicked only ripe cherries through multiple passes on the same trees (Eerie Coffee Company, 2024). These rigorous selection standards are essential in maintaining the quality of coffee, as they directly affect the final cup's aroma, flavor, and overall sensory profile.

Harvesting and pre-processing of coffee cherries

The first step of the coffee processing involved harvesting and selecting fully ripe, red coffee cherries to achieve high-quality coffee beans. The cherries were carefully handpicked they were placed in baskets to prevent bruising before transporting them to the experimental area in Banisilan, Cotabato. To ensure only the best cherries were used, the selection process started with washing the cherries twice using clean water to remove dirt and foreign materials. Low-density floaters, black, dry, or damaged cherries, which indicate poor quality, were discarded, while only dense, high-quality cherries were selected for fermentation. After sorting, the cherries were de-pulped using a machine that gently removed the outer fruit flesh (pulp), leaving the beans encased in their parchment layer, ready for further processing.

Yeast preparation, fermentation, and post-fermentation processing

The preparation of yeast (*Saccharomyces cerevisiae*) involved rehydrating 150 grams of yeast in 3 liters of potable water at an ambient drinking water temperature of 15–37°C. The rehydration was done in a clean bucket, where the yeast was suspended in water and stirred gently to break up clumps. After 10 minutes, the mixture was stirred again to ensure complete dissolution. After 20 minutes, the yeast suspension was introduced into the fermentation tank containing 27 kilograms of pulped coffee beans, ensuring even distribution. Another 27 kilograms of pulped beans were set aside, with 3-kilogram portions used as the untreated (no yeast). The pulped coffee bean was fully submerged, with no more than 1 cm of water above the coffee mass to maintain optimal fermentation conditions (Lallemand Inc., 2019). Once the yeast was applied, fermentation proceeded based on the designated treatment combinations. The pulped coffee was placed inside clean cellophane bags, sealed with rubber bands for anaerobic fermentation, and stored in a clean, dark room. Fermentation naturally progressed according to specific treatment conditions, and 300-gram samples were collected post-fermentation for microbial profiling (Bressani *et al.*, 2021). Following fermentation, the coffee beans were thoroughly washed to remove residual mucilage, using multiple rinses with clean water until the beans were free of any sticky residue.

The washed beans were then transferred to a greenhouse for drying, where they were evenly spread out and dried using artificial dryers until they reached a moisture content of 11–12%. The drying process took place on raised beds, depending on climate conditions and available resources, as proper drying is crucial to prevent mold growth and ensure uniform drying. At the end of the drying phase, 300-gram samples were taken for chemical analysis and microbial profiling (Bressani *et al.*, 2021). Once dried, the beans underwent sorting, grading, and hulling, where the parchment or hull was removed, and the beans were classified based on size, density, and color. Defective beans were eliminated to ensure consistency and maintain high-quality standards.

Experimental design and treatment

The study was conducted using a factorial experiment laid out in a Completely Randomized Design (CRD) with two factors: (a) yeast application, fermented with *Saccharomyces cerevisiae* and without yeast, and (b) fermentation duration, 24 hours, 48 hours, and 72 hours. Each treatment combination was replicated three times. Factor A included two levels: A1 – with yeast fermentation and A2 – without yeast fermentation. Factor B consisted of three levels based on fermentation duration: B1 – 24 hours, B2 – 48 hours, and B3 – 72 hours. The treatment combinations were as follows: A1B1 – Arabica coffee fermented with yeast for 24 hours, A1B2 – Arabica coffee fermented with yeast for 48 hours, A1B3 – Arabica coffee fermented with yeast for 72 hours, A2B1 – Arabica coffee fermented without yeast for 24 hours, A2B2 – Arabica coffee fermented without yeast for 48 hours, and A2B3 – Arabica coffee fermented without yeast for 72 hours.

Data analysis

The various data collected were subjected to the Statistical Tool for Agricultural Research (STAR) through Analysis of Variance (ANOVA). A comparison of means was analyzed using Tukey's Honestly Significant Difference test.

Results

Total soluble solids

The mean total soluble solids (TSS) of *Coffea arabica* var. Bourbon was significantly affected by the presence or absence of yeast, fermentation duration, and their interaction. Samples without yeast (25.40^{a}) had significantly higher TSS than those with yeast (20.67^{b}). Among fermentation durations, 72 hours (24.32^{a}) resulted in the highest TSS, followed by 48 hours (22.14^{b}) and 24 hours (21.98^{b}). The interaction between yeast and fermentation duration showed significant differences, with the highest TSS observed in A2B3 (without yeast, 72 hours) at 26.18^{a}, and the lowest in A1B1 (with yeast, 24 hours) at 19.28^{f}, indicating that both factors interactively influenced TSS levels (Table 1).

Table 1. The mean total soluble solids (TSS) of *Coffea arabica* var. Bourbon, fermented with and without yeast with different fermentation durations, was analyzed at the Department of Science and Technology (DOST) after drying to 11% moisture content

Treatment	Mean (TSS)
A – With and without yeast	
A1 – With yeast	20.67^{b}
A2 – Without yeast	25.40^{a}
F-Test	**
B – Fermentation duration	
B1 – 24 hours of fermentation	21.98^{b}
B2 – 48 hours of fermentation	22.14^{b}
B3 – 72 hours of fermentation	24.32^{a}
F-Test	**
(A × B)	
A1B1	19.28^{f}
A1B2	19.45^{e}
A1B3	22.75^{d}
A2B1	24.81^{c}
A2B2	24.96^{b}
A2B3	26.18^{a}
F-Test	**
CV a (%)	0.1298
CV b (%)	0.0735

This means that a column having the same letter is not significantly different at a 5% level based on Tukey's test. ** – highly significant

Table 2. The mean total titratable acidity (TA) of *Coffea arabica* var. Bourbon, fermented with and without yeast with different fermentation durations, was analyzed at the Department of Science and Technology (DOST) after drying to 11% moisture content

Treatment	Mean (TA)
A – With and without yeast	
A1 – With yeast	0.43^{b}
A2 – Without yeast	0.54^{a}
F-Test	**
B – Fermentation duration	
B1 – 24 hours of fermentation	0.42^{c}
B2 – 48 hours of fermentation	0.49^{b}
B3 – 72 hours of fermentation	0.56^{a}
F-Test	**
(A × B)	
A1B1	0.19^{e}
A1B2	0.44^{c}
A1B3	0.77^{a}
A2B1	0.74^{a}
A2B2	0.54^{b}
A2B3	0.38^{d}
F-Test	**
CV a (%)	5.55
CV b (%)	2.77

This means that a column having the same letter is not significantly different at a 5% level based on Tukey's test. ** – highly significant

Titratable acidity

The mean total titratable acidity (TA) of *Coffea arabica* var. Bourbon was significantly affected by the presence or absence of yeast, fermentation duration, and their interaction. Samples without yeast (0.54^{a}) exhibited significantly higher TA than those with yeast (0.43^{b}) (Table 2).

Fermentation for 72 hours (0.56^{a}) resulted in the highest TA, followed by 48 hours (0.49^{b}) and 24 hours (0.42^{c}). A significant interaction was observed between yeast application and fermentation duration, with the highest TA found in A1B3 (with yeast, 72 hours) at 0.77^{a} and A2B1 (without yeast, 24 hours) at 0.74^{a}, while the lowest was in A1B1 (with yeast, 24 hours) at 0.19^{c}, indicating that both factors interactively influenced titratable acidity.

Table 3. The mean total potential hydrogen (pH) of *Coffea arabica* var. Bourbon, fermented with and without yeast with different fermentation durations, was analyzed at the Department of Science and Technology (DOST) after drying to 11% moisture content

Treatment	Mean (pH)
A – With and without yeast	
A1 – With yeast	5.69^{a}
A2 – Without yeast	5.68^{a}
F-Test	**
B – Fermentation duration	
B1 – 24 hours of fermentation	5.70^{a}
B2 – 48 hours of fermentation	5.69^{ab}
B3 – 72 hours of fermentation	5.67^{b}
F-Test	**
(A×B)	
A1B1	5.71^{a}
A1B2	5.66^{bc}
A1B3	5.71^{a}
A2B1	5.69^{ab}
A2B2	5.73^{a}
A2B3	5.63^{c}
F-Test	**
CV a (%)	0.1435
CV b (%)	0.3366

This means that a column having the same letter is not significantly different at a 5% level based on Tukey's test. ** – highly significant

Potential hydrogen

The mean total potential hydrogen (pH) of *Coffea arabica* var. Bourbon was significantly affected by the presence or absence of yeast, fermentation duration, and their interaction. No significant difference was observed between yeast-treated (5.69^{a}) and untreated samples (5.68^{a}) (Table 3).

Among fermentation durations, 24 hours (5.70^{a}) resulted in the highest pH, followed by 48 hours (5.69^{ab}) and 72 hours (5.67^{b}). A significant interaction was noted, with the highest pH observed in A1B1 (with yeast, 24 hours), A1B3 (with yeast, 72 hours), and A2B2 (without yeast, 48 hours) all at 5.71^{a}–5.73^{a}, while the lowest was in A2B3 (without yeast, 72 hours) at 5.63^{c}, indicating a combined effect of both factors on pH levels.

Table 4. The mean total of colony forming unit (CFU/g) of bacteria after fermentation and drying to 11% moisture content of *Coffea arabica* var. Bourbon, fermented with and without yeast with different fermentation durations, was analyzed at the Microbiology Research Laboratory, College of Veterinary Medicine, CMU

Treatment	Mean CFU/g after fermentation	Mean CFU/g after drying
A – With and without yeast		
A1 – With yeast	49^{a}	301.44^{b}
A2 – Without yeast	39.67^{b}	404.67^{a}
F-Test	**	**
B – Fermentation duration		
B1 – 24 hours of fermentation	51^{a}	230.67^{b}
B2 – 48 hours of fermentation	45.5^{b}	29^{c}
B3 – 72 hours of fermentation	27.5^{c}	799.50^{a}
F-Test	**	**
(A × B)		
A1B1	47^{b}	398.33^{c}
A1B2	45^{b}	34^{e}
A1B3	37^{c}	472^{b}
A2B1	55^{a}	63^{d}
A2B2	46^{b}	$24e^{f}$
A2B3	18^{d}	1127^{a}
F-Test	**	**
CV a (%)	2.21	0.4951
CV b (%)	5.32	0.8756

This means that a column having the same letter is not significantly different at a 5% level based on Tukey's test. ** – highly significant

Colony forming unit (CFU/g) after fermentation and drying

The mean CFU/g of bacteria after fermentation was significantly higher in coffee fermented with yeast

(49) than without yeast (39.67). However, after drying, samples without yeast had significantly higher CFU/g (404.67) than those with yeast (301.44). Among fermentation durations, 24 hours yielded the highest CFU/g after fermentation (51), while 72 hours resulted in the lowest (27.5); after drying, the highest CFU/g was found in the 72-hour treatment (799.50), and the lowest in 48 hours (29). Significant interaction effects between yeast presence and fermentation duration were observed: the highest bacterial load after fermentation was in A2B1 (55), and after drying, in A2B3 (1127), while the lowest values were recorded in A2B3 (18) after fermentation and A2B2 (24) after drying.

Discussion

Total soluble solids

The interaction between yeast application and fermentation duration significantly influenced the total soluble solids (TSS) in *Coffea arabica* var. Bourbon. Yeast-fermented treatments (A1B1, A1B2, A1B3) consistently exhibited lower TSS compared to their non-yeast counterparts (A2B1, A2B2, A2B3), indicating that the presence of yeast accelerates the breakdown of sugars and other soluble compounds during fermentation. This outcome supports previous findings by Silva *et al.* (2019) and Vilela *et al.* (2010), who reported that yeast-driven fermentation enhances sugar metabolism, resulting in lower residual TSS over time. The progressive increase in TSS observed in non-yeast treatments further suggests limited sugar utilization under spontaneous fermentation, highlighting the efficiency of yeast in modulating solute concentrations.

The elevated TSS in non-yeast treatments may be attributed to the slower activity of indigenous microbes, leading to the accumulation of unfermented solutes. This observation aligns with Wang *et al.* (2020), who found that spontaneous fermentation results in higher residual sugars compared to yeast-controlled processes. Meanwhile, the consistent reduction of TSS in yeast-fermented samples underscores the metabolic role of yeast in utilizing soluble solids as energy sources, as described by Zhang *et al.* (2020) and Visser *et al.* (1990). Additionally, the ability of specific yeast strains, such as *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*, to regulate sugar and acid dynamics during fermentation, highlighted by Taillandier *et al.* (2014), may contribute to more predictable physicochemical changes and influence sensory quality in the final product.

Titratable acidity

The analysis of variance (ANOVA) for titratable acidity (TA) in *Coffea arabica* var. Bourbon revealed highly significant effects from yeast application and fermentation duration, as well as their interaction. This indicates that the impact of fermentation time on TA levels is closely dependent on whether yeast is applied. Results showed a clear contrast in TA dynamics between yeast-fermented (A1B1, A1B2, A1B3) and non-yeast-fermented treatments (A2B1, A2B2, A2B3). Specifically, non-yeast treatments demonstrated a decreasing trend in TA over extended fermentation, with higher acidity observed early (A2B1) and reduced levels by the longest duration (A2B3). This decline in acidity may reflect acid consumption by native microbial communities during spontaneous fermentation, a phenomenon noted in studies like that of Silva *et al.* (2013).

In contrast, yeast-fermented treatments exhibited an increasing trend in TA with longer fermentation. The lowest acidity was observed at the shortest duration (A1B1), while the highest occurred at the longest (A1B3), suggesting that yeast activity contributes to acid generation over time. This supports findings by Silva *et al.* (2019) and Wang *et al.* (2020), who associated extended yeast fermentation with enhanced organic acid production. The rise in TA in yeast-inoculated samples may be attributed to yeast species like *Saccharomyces cerevisiae*, which metabolize sugars into acids such as citric and malic acids (Zhang *et al.*, 2020). Furthermore, the interaction effect highlights that early in fermentation; non-

yeast treatments had greater TA than yeast treatments, but this relationship reversed by the final fermentation stage. Such a shift underscores yeast's capacity to drive acid accumulation over time (Taillandier *et al.*, 2014), possibly in collaboration with lactic acid bacteria through synergistic metabolic processes (Vilela *et al.*, 2010). These findings reinforce the importance of optimizing fermentation parameters to modulate acidity according to desired sensory and quality outcomes in fermented products like coffee.

Potential hydrogen

The pH of coffee is a key determinant of its flavor and acidity, and this study showed that both yeast addition and fermentation duration significantly influenced the pH of *Coffea arabica* var. Bourbon.

Yeast-treated samples (A1) maintained slightly higher and more stable pH values compared to non-yeast treatments (A2), supporting the role of *Saccharomyces cerevisiae* in moderating organic acid production and buffering acidification (Silva *et al.*, 2019; Zhang *et al.*, 2020). Controlled yeast fermentation likely limited excessive acid buildup, contributing to more consistent pH outcomes and aligning with findings by Wang *et al.* (2020) and Taillandier *et al.* (2014).

Longer fermentation times, particularly at 72 hours (B3), led to lower pH, indicating increased microbial activity and acid accumulation (Silva *et al.*, 2013). The interaction of yeast and time revealed greater pH stability in yeast-treated samples (A1B1, A1B3), while spontaneous fermentations without yeast (A2B3) showed the lowest pH, reflecting unregulated microbial metabolism (Vilela *et al.*, 2010; Visser *et al.*, 1990). Interestingly, A2B2 recorded the highest pH, possibly due to ammonium ion release during microbial shifts (Silva *et al.*, 2019). Overall, yeast fermentation helped stabilize pH and may enhance flavor balance and sensory consistency, especially important in maintaining quality across coffee batches (Zhang *et al.*, 2020).

Colony forming unit (CFU/g) of bacteria after fermentation and after drying

The bacterial colony-forming units (CFU) after fermentation were significantly influenced by yeast addition and fermentation duration, as well as their interaction. The presence of yeast (A1) generally supported higher CFU counts at earlier stages, particularly at 24 hours (A1B1), suggesting that yeast may stimulate early bacterial activity (Chen and Zhao, 2012). However, CFU values declined in both treatments as fermentation time increased, with the lowest count observed at 72 hours without yeast (A2B3), likely due to acidification and nutrient depletion (Smith and Jones, 2010; Johnson and Brown, 2011). The yeast-treated group showed a more gradual decrease in CFU (A1B1 to A1B3), indicating that yeast may offer a stabilizing environment for bacterial survival during the initial fermentation stages (Lee *et al.*, 2011).

After drying, a distinct shift in bacterial dynamics was observed. Without yeast (A2), CFU counts sharply increased with fermentation duration, peaking at A2B3, the highest among all treatments. In contrast, yeast-treated samples (A1) showed moderate CFU counts post-drying, with A1B3 notably lower than its non-yeast counterpart. This indicates that while yeast may enhance bacterial growth early on, its presence might limit bacterial survival post-drying, possibly due to competition or metabolic by-products (Lee *et al.*, 2011; Johnson and Brown, 2013). The significant interaction suggests that the benefits of yeast on bacterial viability are time-dependent, supportive during early fermentation, but less favorable after prolonged fermentation and drying. These findings reinforce the complexity of microbial interactions and underscore the need for precise control in fermentation processes to optimize microbial outcomes (Smith and Jones, 2012; Chen and Zhao, 2012).

Conclusion

The study reveals that yeast addition and fermentation duration significantly affect the total soluble solids (TSS), titratable acidity (TA), pH, and

microbial profile of fermented Arabica coffee. Yeast presence notably reduced TSS over time, indicating active sugar metabolism, while also increasing TA, suggesting enhanced organic acid production that improves coffee quality. pH declined primarily due to fermentation duration rather than yeast addition. Microbial analysis showed that yeast initially promoted bacterial growth, but prolonged fermentation and drying led to reduced bacterial counts, especially in yeast-treated samples, highlighting yeast's role in shaping a more competitive and selective microbial environment.

Recommendations

Optimizing fermentation time is crucial for achieving the desired coffee quality, as yeast fermentation influences sugar metabolism and acidity. Extending fermentation beyond 48 hours enhances acidity and reduces residual sugars, while a shorter 24-hour process results in a sweeter profile. Controlled yeast fermentation, particularly with *Saccharomyces cerevisiae*, ensures consistent physicochemical changes, improving flavor and maintaining stable TSS and acidity levels. Monitoring microbial dynamics during fermentation and drying is essential for safety and quality control, with pH management and optimal drying conditions helping prevent unwanted microbial growth. Further research should explore yeast strain interactions with native microbes and the influence of environmental factors like temperature and oxygen availability on fermentation outcomes.

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