Junior Science Communications
Faculty of Applied Sciences, UiTM Shah Alam
https://journal.uitm.edu.my/ojs/index.php/JSC

E-ISSN 2637-0689
Vol 69 2026

Colloquium on Applied Sciences 1 2026
19-22 January 2026, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Dual Culture Fermentation of Arabica Coffee Using *Monascus* sp. and *Saccharomyces cerevisiae*: Growth Behaviour and Nutritional Impact

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Structured Abstract

Background: Coffee fermentation is traditionally applied during post-harvest processing to improve flavour development; however, its potential to enhance functional and bioactive properties remains underexplored. Advanced microbial fermentation using selected starter cultures offers opportunities to enrich coffee with health-promoting metabolites. Baker's yeast *Saccharomyces cerevisiae* and red rice fungus *Monascus* sp. are well-established fermentative microbes known for their capacity to generate bioactive compounds. However, studies investigating the use of these strains in a dual culture fermentation system for coffee processing have not yet been reported. This study aimed to evaluate the growth behaviour and nutritional effects of these microbial strains on Arabica coffee.

Methods: Solid state fermentation (SSF) was performed using ground Arabica coffee beans as the substrate employing dual culture system of *Monascus* sp. and *S. cerevisiae* under controlled conditions. Microbial growth was monitored via colony expansion, pigment production, and viable cell counts, while sugar utilisation was assessed using Brix measurements. Methanolic extracts from day 0, 3, and 6 were analysed for total phenolic content (TPC) using the Folin–Ciocalteu assay and metabolite profiling using gas chromatography-mass spectrometry (GC–MS). Unfermented and single culture fermentation systems were used as controls.

Results: Both *Monascus* sp. and *Saccharomyces cerevisiae* exhibited consistent growth under single and dual culture fermentation conditions, with no significant difference observed in the dual culture system ($p < 0.05$), indicating minimal antagonistic interaction. Complete sugar depletion occurred during early fermentation, followed by a slight increased to 0.3–0.5 °Bx by day 6, reflecting active microbial metabolism. TPC increased by 21.5% in single culture *S. cerevisiae* and by 10.6% in dual culture from day 3 to day 6, indicating enhanced antioxidant related properties. Metabolite profiling identified fermentation associated compounds including hexadecanoic acid and oleic acid, reported for antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and cardiovascular benefits.

Conclusion: Dual culture SSF using *Monascus* sp. and *S. cerevisiae* can be successfully applied to Arabica coffee beans without compromising microbial growth. This approach enhanced antioxidant related properties and modified the metabolite profile of coffee, highlighting its potential for developing functional or postbiotic coffee products.

Keywords: Arabica coffee, solid state fermentation, *Monascus* sp., *Saccharomyces cerevisiae*, dual culture fermentation system

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