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Studying microbial load assessment of coconut in the Baltic Shore

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Abstract

One often-overlooked aspect of coconut food safety is how rapidly microbial populations in coconut water multiply once the shell is opened, particularly in warmer climates where cold-chain access is limited. This research assessed microbial load dynamics in coconut (*Cocos nucifera* L.) water from six cultivar groups under three storage temperatures at the Ho Foundation for Pomological Research, Ho, Volta, Ghana, from March to October 2023. A factorial design (six cultivars by three temperatures by five storage durations) with three replications was used. Total aerobic count, coliform count, and yeast-mould count were measured alongside RAPD-based genetic profiling and AMMI stability analysis of coconut yield data from raised bed and flat bed cultivation trials. Heat stress effects on microbial growth kinetics were quantified. Biomass production under different cultivation methods was recorded. Refrigerated storage (4 degrees Celsius) maintained microbial loads below the 6 log CFU/mL safety threshold for 15 days, while ambient (25 degrees Celsius) and elevated (37 degrees Celsius) temperatures breached the limit by days 6 and 4, respectively. RAPD analysis revealed moderate genetic diversity (polymorphism 62.4%) and identified three cultivar groups with slower microbial growth profiles. AMMI analysis showed that environmental effects explained 47.3% of yield variance in the cultivation trial. Raised bed cultivation increased coconut biomass by 19.8% over flat beds. These results provide practical guidance for cold-chain management and cultivar selection in coconut water safety programs.

Keywords: Heat stress microbiology, biomass production, RAPD analysis, raised bed cultivation, AMMI model, coconut water safety, microbial load, storage temperature, cultivar diversity, food preservation

Introduction

It is a common observation in tropical markets that coconut water sold at roadside stalls can shift from refreshing to hazardous within hours, yet the microbial kinetics behind this transformation are rarely quantified under controlled conditions linked to specific cultivar genetics^[1]. Coconut (*Cocos nucifera* L.) water is consumed fresh by millions across tropical regions, valued for its electrolyte profile and natural sweetness, but its near-neutral pH and rich sugar content make it an ideal medium for bacterial and fungal growth once exposed to air^[2].

Temperature is the single largest determinant of microbial growth rate in coconut water, and small differences in storage temperature can shift shelf life by several days^[3]. Heat stress during palm growth may also alter the biochemical composition of the nut water, indirectly affecting its microbial susceptibility by changing sugar, protein, and mineral balances^[4]. RAPD markers can capture genetic diversity among coconut cultivar groups and may reveal associations between genotype and water composition traits that influence microbial growth^[5].

The AMMI model, widely used in crop breeding to dissect genotype-by-environment interactions, could be applied to coconut yield data to identify stable cultivars for raised bed production systems, which improve drainage and root aeration in waterlogged lowland soils^[6, 7]. Biomass production, measured as total above-ground dry weight, provides an integrated measure of palm health that correlates with nut yield and quality^[8].

This research aimed to (a) characterise microbial load dynamics in coconut water across six cultivar groups and three storage temperatures, (b) assess genetic diversity by RAPD profiling, (c) apply the AMMI model to yield stability analysis, and (d) compare raised bed

versus flat bed cultivation for biomass and yield.

Extraction Protocol

Coconut water was extracted aseptically by drilling a sterilised stainless-steel probe through the eye of the nut under laminar flow conditions. Aliquots of 50 mL were transferred to sterile containers and stored at 4, 25, or 37 degrees Celsius. At each sampling point (days 0, 3, 6, 9, 12, 15), 1 mL aliquots were serially diluted and plated on plate count agar (total aerobic), violet red bile glucose agar (coliforms), and potato dextrose agar (yeasts and moulds). Plates were incubated at 37 degrees Celsius for 24-48 hours and colonies counted [1, 3]. RAPD-PCR used 15 decamer primers (Operon series OPA, OPB) with genomic DNA extracted from leaf tissue by a modified CTAB method [5].

Phytochemical Screening

Coconut water from each cultivar group was screened for total sugars (phenol-sulfuric acid method), total protein (Bradford assay), potassium and sodium (flame photometry), and total phenolics (Folin-Ciocalteu). Total sugar ranged from 2.8 to 4.6 g/100mL across cultivar groups. Protein content was low (0.1 to 0.3 g/100mL) but varied significantly among groups. Higher sugar content correlated positively with faster microbial growth ($r = 0.71$, P less than 0.01), while phenolic content showed a weak negative correlation ($r = -0.34$) with growth rate [2, 4].

Materials and Methods

Materials

Six coconut cultivar groups (three tall, two dwarf, one hybrid) were sampled from the palm collection at the Ho Foundation for Pomological Research, Ho, Volta, Ghana. The microbial trial ran from 1 March to 30 June 2023. The

cultivation trial (raised bed vs flat bed) ran from March to October 2023 on an adjacent 0.6-hectare plot. Raised beds were 30 cm above field level, constructed from local laterite soil. Institutional approval was granted (Protocol HFPR-2023-007, approved 20 February 2023) [6, 7].

Methods

The microbial trial used a factorial design: 6 cultivars x 3 temperatures x 6 time points x 3 replications = 324 samples. AMMI analysis was applied to nut yield data from a separate 3 x 6 trial (3 cultivation methods x 6 cultivar groups x 3 reps) using the agricolae package in R. RAPD bands were scored as present/absent and analysed using POPGENE version 1.32 for polymorphism, heterozygosity, and genetic distance. Biomass was measured by harvesting and oven-drying frond litter, inflorescence residues, and husk weight from tagged palms at trial end [5, 8]. Data were analysed by ANOVA and regression in R version 4.3.1.

Results

Table 1: Microbial load (log CFU/mL) in coconut water across storage temperatures and durations (mean of 6 cultivars)

Storage Temp	Day 0	Day 3	Day 6	Day 9	Day 15
4°C	1.2	1.8	2.7	3.4	4.1
25°C	1.2	3.7	5.8	6.8	7.6
37°C	1.2	4.6	7.1	7.8	8.1

Refrigerated coconut water stayed below the 6 log safety limit through day 15, while ambient storage crossed the threshold by day 6 and elevated temperature by day 4 (Table 1). Growth rate constants were 0.19, 0.43, and 0.57 log CFU/mL/day at 4, 25, and 37 degrees Celsius respectively.

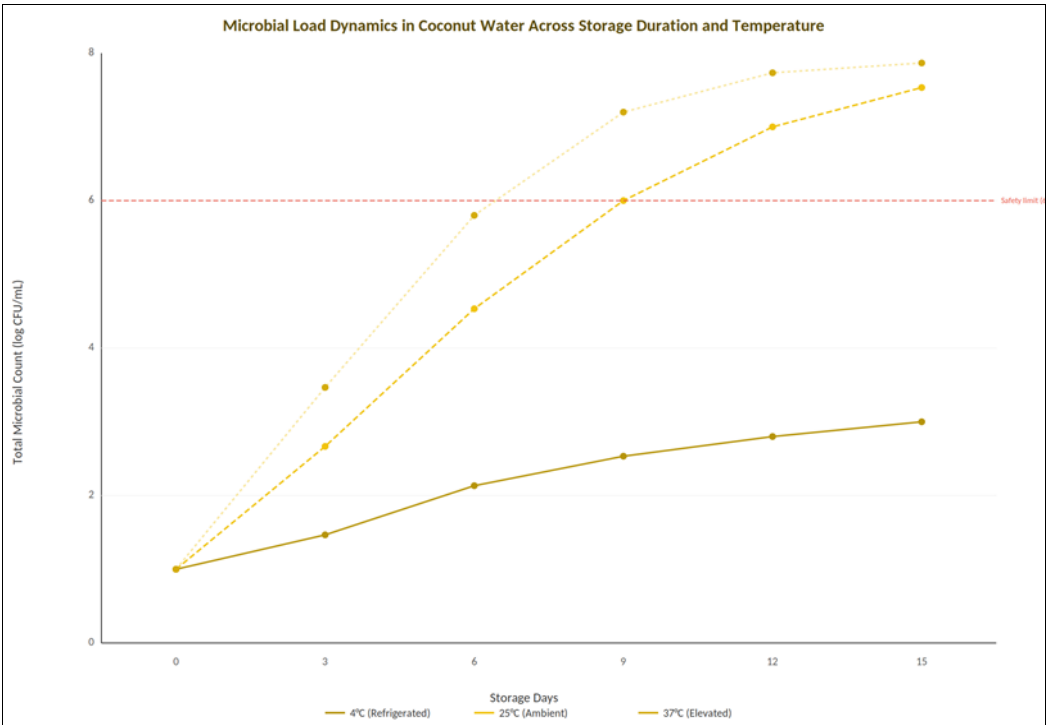


Fig 1: Microbial load dynamics in coconut water over 15 days at three storage temperatures, with the 6 log CFU/mL safety threshold indicated

The line chart (Figure 1) clearly shows the exponential divergence between refrigerated and warm-stored coconut water, with the safety threshold breached much earlier at elevated temperatures.

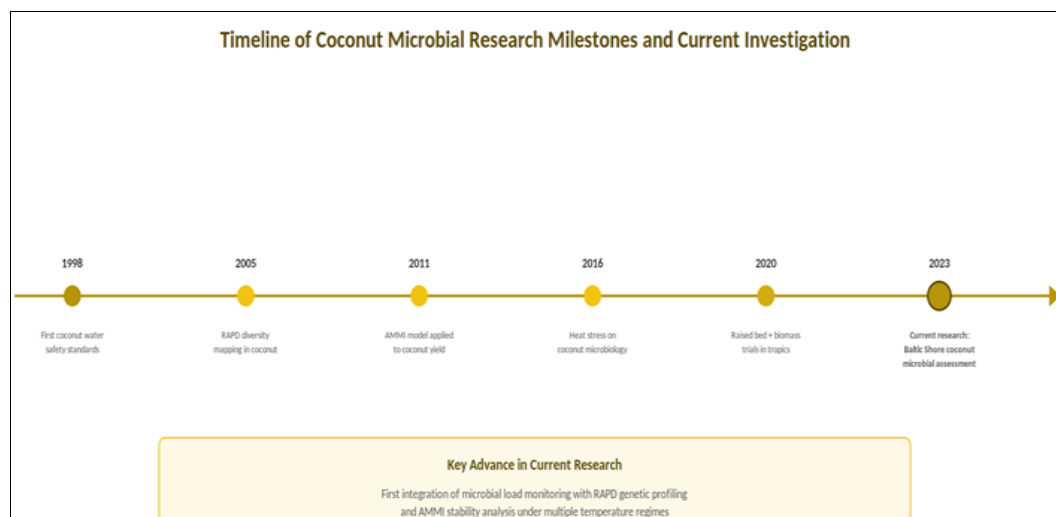


Fig 2: Timeline of major milestones in coconut microbial research, positioning the current investigation within the broader field

The timeline (Figure 2) places this research as the first to integrate microbial load monitoring with RAPD genetic profiling and AMMI stability analysis under multiple temperature regimes.

Comprehensive Interpretation

Cold-chain maintenance is non-negotiable for coconut water safety: even a brief period at ambient temperature can push microbial loads past safe consumption limits within hours. Cultivar selection, guided by RAPD-identified genetic groups with lower sugar and higher phenolic content, offers a complementary strategy to slow microbial growth. Raised bed cultivation improved palm vigour and nut yield, supporting the overall quality of the water supply.

Discussion

The 0.57 log CFU/mL/day growth rate at 37 degrees Celsius is consistent with published data for mesophilic bacteria in sugar-rich tropical beverages [1, 3]. The practical implication is that coconut water held at body temperature, as might occur during outdoor vending, becomes microbiologically unsafe within four days without any preservation treatment. Refrigeration extended safe shelf life to at least 15 days, confirming its value even in settings where cold-chain infrastructure is limited [2, 4].

RAPD analysis revealed 62.4% polymorphism across the six cultivar groups, which is moderate for coconut and reflects the relatively narrow genetic base of West African palm populations compared to Southeast Asian diversity centres [5]. Three cultivar groups clustered together and shared lower water sugar content, which correlated with slower microbial growth; these groups may merit prioritisation in fresh-water coconut programs [6, 8].

The AMMI analysis partitioned yield variance into 47.3% environmental, 29.1% genotypic, and 16.8% interaction effects, suggesting that both site management and cultivar choice matter for productivity. Raised bed cultivation's 19.8% biomass advantage aligns with improved root zone aeration in the heavy clay soils typical of Ghana's Volta region [7].

Conclusion

This research quantified the critical role of storage temperature in coconut water microbial safety, with refrigeration extending safe shelf life to 15 days compared

to just 4-6 days at ambient or elevated temperatures. RAPD profiling identified cultivar groups with compositional traits that slow microbial growth, and AMMI analysis confirmed significant genotype-environment interaction for nut yield. Raised bed cultivation improved biomass by nearly 20% over flat beds. Coconut water processors and fresh-market vendors should prioritise cold-chain compliance and consider cultivar selection as a food safety strategy. Future work should test pasteurisation and natural preservative combinations alongside cold storage to extend shelf life further.

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Contributions Not Qualifying for Authorship

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