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Research paper

Antioxidant Activity and Antibacterial Activity of Fermented Coconut Water by L. Plantarum and L. Fermentum

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Abstract

Coconut water (CW) is a nutritious natural beverage, rich in vitamins, minerals, carbohydrates, proteins, and enzymes, and is low in fat and calories. It has been associated with health benefits such as antidiabetic and antihyperlipidemic effects. Fermentation of CW may enhance its bioactivity, particularly its antioxidant properties and inhibition of starch-hydrolyzing enzymes. This study investigated the antioxidant and antibacterial activities of fermented coconut water (FCW) using Lactobacillus plantarum ATCC 8014 and Lactobacillus fermentum ATCC 14932. After 48 hours of fermentation, the total phenolic content (TPC) increased significantly to 18.91 mg/mL GAE in FCW with 10% allulose and to 25.23 mg/mL GAE in FCW with 10% xylitol. Both fermented samples showed improved ABTS and DPPH radical scavenging activity, although their hydroxyl radical scavenging capacity was lower than that of unfermented CW. Antibacterial assays revealed that FCW with 10% xylitol inhibited the growth of Staphylococcus aureus ATCC 25523, Escherichia coli ATCC 10536, and multiple Salmonella strains. In contrast, FCW with 10% allulose showed no antibacterial activity. These findings suggest that xylitol-enriched FCW may serve not only as a sustainable beverage but also as a natural antioxidant and antibacterial agent for potential applications in functional food, gut health, and food preservation systems.

Keywords: *Antibacterial Activity; Antioxidant Activity; Fermented Coconut Water; Lactobacillus Fermentum; Lactobacillus Plantarum.*

1. Introduction

Coconut (Cocos nucifera L.) consists of three primary components: the meat, the water, and the shell. While the shell is often processed into activated carbon, the water, particularly from mature coconuts, is frequently discarded during coconut milk production, contributing to significant food waste (Naik et al., 2023). A coconut typically matures over 11–12 months (Patrick & Offer, 2001), during which its water transforms from a sour, low-sugar solution to a sweeter, nutrient-dense liquid as sugars accumulate and endosperm develops. Coconut water (CW) contains essential nutrients such as glucose, fructose, sucrose, malic acid, citric acid, amino acids, vitamins B and C, and minerals including sodium, potassium, calcium, magnesium, zinc, and iron (Alexia et al., 2012; Thorburn et al., 2020; Jean et al., 2009; Matsui et al., 2007; Mukund et al., 2021).

Beyond these nutrients, CW also harbors bioactive enzymes like polyphenol oxidase (PPO) and peroxidase (POD), as well as growth-promoting compounds such as sorbitol and myo-inositol (Angel, 2023; Chathuri et al., 2018). Its naturally low-fat and calorie profile makes it an increasingly popular plant-based sports drink, especially among health-conscious and lactose-intolerant consumers. Multiple studies have attributed antioxidant, antidiabetic, antihyperlipidemic, and cardioprotective properties to CW (Busarakorn et al., 2016; Aluísio et al., 2009; Preetha et al., 2013; Sandhya & Rajamohan, 2008; Rini et al., 2014; Alleyne et al., 2005).

Despite these advantages, the large-scale industrial use of mature CW remains limited, leading to economic inefficiency and environmental concerns. In response, fermentation has emerged as a promising food engineering strategy for valorizing CW into high-value functional beverages. Fermentation with lactic acid bacteria (LAB), such as Lactobacillus plantarum and L. delbrueckii, has been shown to improve both physicochemical and bioactive properties of plant substrates (Luying et al., 2019). Moreover, co-culture fermentations have demonstrated superior outcomes compared to single strains in enhancing antioxidant activity and improving microbial safety (Shana et al., 2015). In addition to LAB, other microbial systems such as yeast-based or symbiotic fermentations (e.g., kombucha-style) have also been explored for coconut-derived beverages, albeit less frequently. These systems emphasize the interdisciplinary potential of fermented CW across

microbiology, food technology, and public health—particularly in developing shelf-stable, bioactive beverages that could replace artificial preservatives or support gut health.

Recent work by Mindan et al. (2015) and Dhanya et al. (2023) revealed that fermentation enhances CW's antioxidant and anti-uropathogenic activities. Dhanya et al. reported an increase in antioxidant potential from 48.33 ± 2.52% in unfermented CW to 55.12 ± 2.99% in fermented CW (FCW), along with newly observed antibacterial effects. Likewise, Sib et al. (2018) demonstrated increased total phenolic content and radical scavenging activities in CW fermented with Lactobacillus casei.

Building on these findings, the present study investigates the antioxidant and antibacterial activities of CW fermented with a co-culture of Lactobacillus plantarum ATCC 8014 and Lactobacillus fermentum ATCC 14932 (X et al., 2025). This research aims to assess the functional enhancement of CW through fermentation and explore its potential application as a sustainable, health-promoting functional beverage, relevant to both food preservation and preventive health strategies.

2. Materials and Methods

2.1. Preparation of starter culture

Lactobacillus plantarum ATCC 8014 and Lactobacillus fermentum ATCC 14932 were streaked onto MRS agar plates and incubated at 37 °C for 48 hours. A single colony from each plate was transferred into MRS broth and incubated at 37 °C for 18 hours. The cultures were centrifuged at 8000 × g for 15 minutes at 4 °C, washed twice with sterile saline solution (0.85%, w/v), and resuspended in sterile coconut water to achieve a final cell density of approximately 1×10^7 CFU/mL. This suspension was used as the starter culture.

2.2. Coconut water fermentation preparation

Coconuts aged 8–9 months were obtained from a local market near Universiti Putra Malaysia (UPM). After washing, the coconuts were opened using a sterile stainless-steel knife under laminar flow conditions. The fresh coconut water was filtered through four layers of filter paper (125 mm diameter × 100 circles, Whatman, China). Xylitol (10%) and allulose (10%) were each added separately to the coconut water. The mixtures were pasteurized in a water bath at 75 °C for 15 minutes. After cooling, 3% (v/v) co-culture of L. plantarum ATCC 8014 and L. fermentum ATCC 14932 (1:1 ratio) was added to 50 mL of coconut water. Fermentation was carried out at 37 °C for 48 hours.

2.3. DPPH radical scavenging ability

The DPPH radical scavenging activity of FCW and CW was determined with slight modifications based on methods by Sib et al. (2018) and Yu et al. (2021). One milliliter of sample supernatant was mixed with 2 mL of 0.1 mmol/L DPPH methanol solution. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm and recorded as A1. A0 (blank) was obtained by replacing the sample with distilled water. Each sample was tested in triplicate. Fresh CW served as the control. Scavenging activity was calculated as:

$$\text{DPPH scavenging rate (\%)} = [(A0 - A1)/A0] \times 100$$

2.4. ABTS Radical Scavenging Ability

ABTS radical scavenging activity was measured following Busarakorn et al. (2016) and Rainie & Ranjani (2022) with minor modifications. Equal volumes of 7 mmol/L ABTS and 2.45 mmol/L $K_2S_2O_8$ solutions were mixed and allowed to react in the dark at room temperature for 16 hours to generate $ABTS^{\bullet+}$ radicals. The solution was diluted with absolute ethanol to an absorbance of 0.700 ± 0.02 at 734 nm. Then, 0.3 mL of sample supernatant was mixed with 5 mL of the $ABTS^{\bullet+}$ solution and incubated in the dark for 6 minutes at room temperature. Absorbance was measured at 734 nm (A1). A0 was measured using distilled water. Each sample was tested in triplicate. Scavenging activity was calculated as:

$$\text{ABTS scavenging rate (\%)} = [(A1 - A0)/A0] \times 100$$

2.5. Hydroxyl radical scavenging ability

Hydroxyl radical scavenging activity was assessed based on Zhang et al. (2009) and Shao (2019), with slight modifications. The reaction mixture contained 0.5 mL sample supernatant, 1 mL Fe^{2+} solution (6 mmol/L), 1 mL salicylic acid-ethanol solution (6 mmol/L), and 1 mL H_2O_2 (6 mmol/L). After incubation in the dark at 37 °C for 30 minutes, absorbance was measured at 510 nm (A1). A0 was measured using distilled water in place of the sample. A2 was measured by replacing H_2O_2 with distilled water. Each assay was performed in triplicate. Scavenging activity was calculated as:

$$\text{Hydroxyl radical scavenging rate (\%)} = [1 - (A1 - A2)/A0] \times 100$$

2.6. Total phenolic content

TPC was determined using a modified Folin–Ciocalteu method (Sib et al., 2018; Rainie & Ranjani, 2022). Ten milliliters of sample supernatant was mixed with 200 µL Folin–Ciocalteu's reagent. After 3 minutes, 800 µL of 7.5% (w/v) Na_2CO_3 was added, and the mixture was incubated in the dark at room temperature for 2 hours. Absorbance was measured at 765 nm. A calibration curve was prepared using gallic acid (0–8.2 mg/mL), and results were expressed as mg gallic acid equivalents (GAE) per mL. Each sample was analyzed in triplicate.

2.7. Antibacterial activity

The antibacterial activity of FCW was assessed using the agar well-diffusion method. FCW supernatants were centrifuged at 8000 × g for 20 minutes at 4 °C and filtered through a 0.45 µm Millipore filter. Test bacteria included Salmonella ATCC 13311, ATCC 14028, ATCC

13076, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus ATCC 25523, Escherichia coli ATCC 10536, and Listeria monocytogenes. Each strain was cultured overnight in TSB broth at 37 °C, then streaked on nutrient agar and incubated for 24 hours. Colonies were suspended in 0.85% saline and adjusted to an absorbance of 0.08–0.10. Indicator bacteria were spread on Mueller-Hinton agar (MHA) plates using sterile cotton swabs. Wells (5 mm diameter, 5 mm depth) were filled with 100 µL of FCW supernatant. Plates were incubated aerobically at 37 °C for 24 hours. The diameter of the inhibition zone was measured in centimeters.

2.8. Statistical analyses

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between treatments. Results were expressed as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

3. Result and Discussion

3.1. Total phenolic content (TPC)

The total phenolic content (TPC) of fermented coconut water (FCW) increased significantly after 48 hours of fermentation. FCW supplemented with 10% allulose reached 18.91 mg/mL GAE, while FCW with 10% xylitol achieved a higher value of 25.23 mg/mL GAE, compared to 17.78 mg/mL GAE in unfermented coconut water. Phenolic compounds are prominent antioxidants found in plant-based foods (Sib et al., 2018), and previous studies have identified (+)-catechin and (−)-epicatechin in coconut water (Chang & Wu, 2011). The increase in TPC may be attributed to microbial biotransformation during fermentation, which can enhance the solubility or bioavailability of polyphenols (Ghosh et al., 2015; Mindani et al., 2016). Similar improvements have been reported in cereal- and fruit-based substrates fermented with lactic acid bacteria and yeasts (Kantachote et al., 2017; Zhang et al., 2018).
Importantly, phenolic content alone does not determine antioxidant efficacy. Bioactive metabolites and pH changes during fermentation may also influence antioxidant capacity (Kantachote et al., 2017). The observed TPC trends correlate with the antioxidant results described below, supporting the hypothesis that phenolic enhancement contributes to increased radical scavenging.

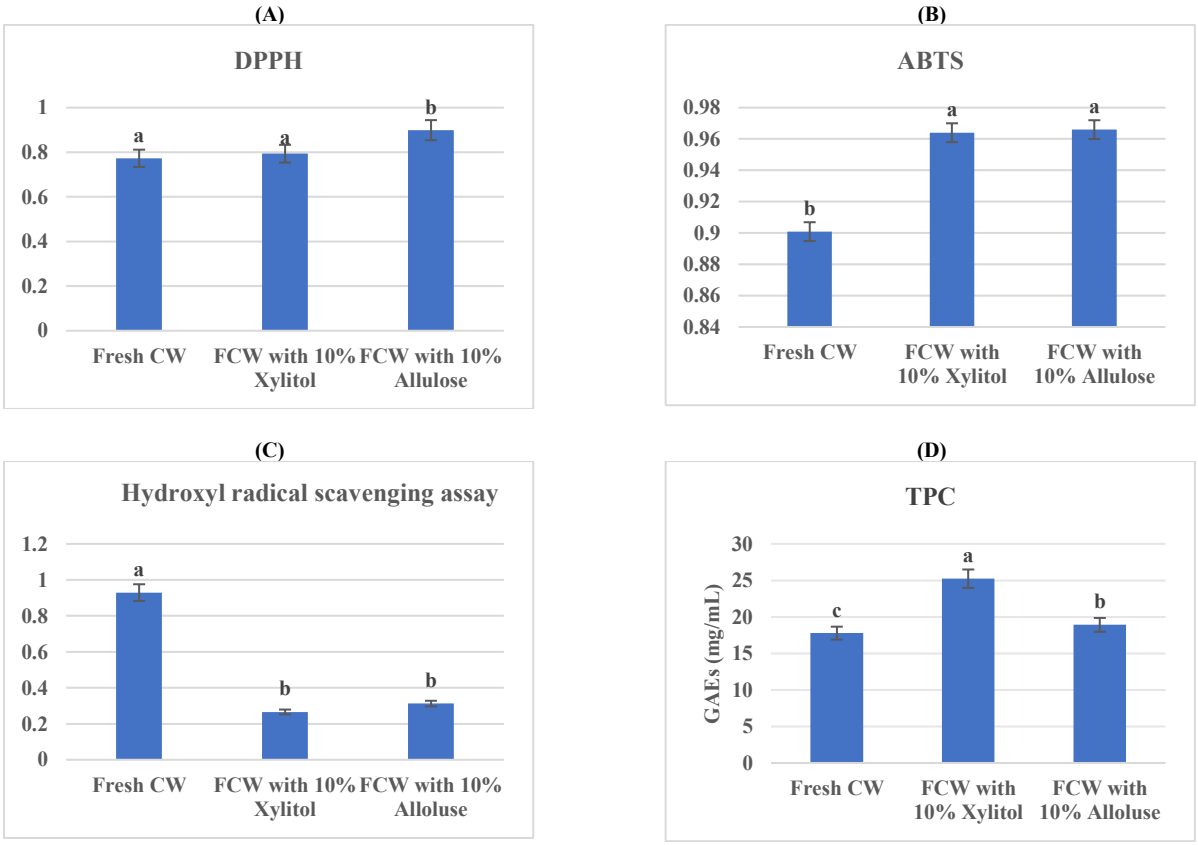

Fig. 1: Functional Properties of Fermented Coconut Water. (A) DPPH Radical Scavenging (%); (B) ABTS Radical Scavenging (%); (C) Hydroxyl Radical Scavenging (%); (D) Total Phenolic Content (Mg GAE/Ml). Error Bars Represent ± SD. Different Superscript Letters Indicate Significant Differences ($P < 0.05$).

3.2. Antioxidant activities

As shown in Figures 1a and 1b, both FCW treatments exhibited enhanced antioxidant activity post-fermentation. ABTS radical scavenging increased from 90.09% in fresh coconut water to 96.60% (allulose) and 96.40% (xylitol). Similarly, DPPH scavenging rose modestly, in line with findings from Yu et al. (2021) and Pianpumepong et al. (2012), who demonstrated improved antioxidant capacity in LAB-fermented substrates. This enhancement is likely due to microbial release or transformation of phenolic compounds and other antioxidant metabolites (Martin et al., 2020).
In contrast, hydroxyl radical scavenging decreased significantly post-fermentation to 31.23% (xylitol) and 26.59% (allulose) (Fig. 1c). This divergence from ABTS/DPPH results may reflect the selective reactivity of different radicals or compound-specific interactions. Prior studies reported variable hydroxyl radical responses depending on coconut maturity, strain selection, and sugar additives (Rachana &

Nikihilesh, 2017). These results emphasize the complex interplay of substrate chemistry, fermentation parameters, and antioxidant assays. Further profiling using HPLC/MS is recommended to identify specific bioactive compounds responsible for these effects.

3.3. Antibacterial activity

FCW supplemented with 10% xylitol demonstrated broad-spectrum antibacterial activity against multiple foodborne pathogens, including S. aureus ATCC 25523, E. coli ATCC 10536, and Salmonella strains ATCC 13311, 14028, and 13076. The largest inhibition zone (0.210 cm) was recorded against Salmonella ATCC 14028, while no inhibition was observed with FCW-allulose (Table 1). These findings suggest xylitol may stimulate the production of antimicrobial metabolites (e.g., bacteriocins, organic acids) or alter pH to unfavorable levels for pathogen survival.

Comparable results were reported by Rachana and Nikihilesh (2017) and Rakshitha et al. (2022), who observed enhanced antibacterial activity in fermented coconut water compared to unfermented controls. The lack of activity in FCW-allulose aligns with reports suggesting sugar type influences LAB metabolism and bioactive production. Notably, both unpasteurized and pasteurized FCW exhibited inhibition zones against pathogens in studies by Olaide et al. (2020), reinforcing fermentation's potential for natural preservation.

Although inhibition zones in this study were smaller than typical antibiotic thresholds, they support FCW's potential as a complementary antimicrobial agent. Future tests against multidrug-resistant and biofilm-forming strains could broaden applicability.

Table 1: Diameter of Inhibition Zones (Cm) For Fermented Coconut Water (FCW) Against Indicator Bacteria

Indicator bacteria	S. aureus ATCC 25523	E. coli ATCC 10536	Salmonella ATCC 13311	Bacillus cereus ATCC 14028	Bacillus subtilis ATCC 13076	Listeria monocytogenes
FCW with 10% xylitol	0.133 ± 0.017^{b}	0.023 ± 0.031^{a}	0.053 ± 0.087^{a}	0.210 ± 0.029^{c}	ND	ND
FCW with 10% allulose	ND	ND	ND	ND	ND	ND

Note: ND = No inhibition zone detected.

Superscript letters indicate significant differences ($p < 0.05$) between treatments.

3.4. Summary of key findings

This study demonstrated that fermented coconut water (FCW) supplemented with 10% xylitol exhibited superior functional properties compared to allulose-supplemented FCW. Specifically, the xylitol formulation yielded higher total phenolic content (TPC) and broader antibacterial activity against several foodborne pathogens. Both fermentation treatments significantly enhanced the antioxidant capacity of coconut water, as evidenced by improved ABTS and DPPH radical scavenging activities, with FCW-xylitol showing slightly greater efficacy. However, a reduction in hydroxyl radical scavenging activity was observed post-fermentation in both treatments, highlighting a potential area for further investigation. The enhanced antibacterial effect associated with xylitol may be attributed to its influence on microbial metabolism or the production of bioactive compounds during fermentation.

3.5. Limitations

This study did not quantify LAB viability post-fermentation, which could influence antioxidant or antimicrobial outcomes. Additionally, variability in coconut maturity and composition may affect reproducibility. Future work should examine industrial scalability, including continuous fermentation and shelf-life assessments. HPLC/MS metabolite profiling and gut microbiota interaction studies are also recommended to evaluate FCW's broader health applications.

3.6. Future directions and scientific implications

The promising antioxidant and antimicrobial properties of xylitol-enriched fermented coconut water (FCW) highlight its potential as a functional food or natural preservative. However, before translation to a large-scale application, several scientific and technical aspects must be addressed.

From a scalability perspective, future research should optimize industrial fermentation parameters, including fermentation vessel design, oxygen levels, inoculum concentration, and downstream processing. These steps are critical to ensure consistent microbial viability, phenolic retention, and product stability during shelf life.

For bioactive characterization, advanced metabolomic techniques such as HPLC or LC-MS should be employed to identify and quantify individual phenolic compounds and secondary metabolites produced during fermentation. This will help clarify the mechanisms behind the enhanced antioxidant and antibacterial effects observed in the xylitol formulation.

Application-wise, FCW could be explored as an ingredient in antimicrobial packaging materials or as a component in probiotic formulations aimed at improving gut health. The synergy between LAB strains and bioactive metabolites could offer dual benefits—pathogen inhibition and microbiota support.

Additionally, technical extensions may include testing FCW efficacy against biofilms and multidrug-resistant (MDR) bacterial strains, given the growing relevance of natural antimicrobials in food safety and clinical contexts. Investigating these applications could broaden the interdisciplinary value of FCW, bridging food science, microbiology, and public health.

4. Conclusion

This study demonstrated that Lactobacillus plantarum ATCC 8014 and Lactobacillus fermentum ATCC 14932 can effectively ferment coconut water (CW), enhancing its functional potential. Fermentation significantly elevated the total phenolic content (TPC), particularly in the xylitol-supplemented formulation (25.23 mg GAE/mL), which corresponded with stronger ABTS and DPPH radical scavenging activities. This underscores a phenolic-driven mechanism for improved antioxidant capacity. However, hydroxyl radical scavenging activity declined post-fermentation, diverging from prior literature and warranting further exploration into substrate maturity, microbial metabolism, or matrix interactions.

Notably, FCW with 10% xylitol exhibited measurable antibacterial activity against Staphylococcus aureus, Escherichia coli, and multiple Salmonella strains, unlike its allulose counterpart. This suggests that xylitol may play a role in modulating microbial metabolite synthesis or altering physicochemical properties that suppress pathogenic growth.

While these results highlight the potential of xylitol-enriched FCW as a functional beverage with dual antioxidant and antimicrobial properties, further research is recommended. Future studies should include profiling of bioactive metabolites (e.g., via HPLC/MS), assessment of LAB viability post-fermentation, and exploration of scalability under industrial conditions. Moreover, potential applications in food preservation, gut health, or antimicrobial film development could be explored to expand its interdisciplinary relevance.

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