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Evaluation of palm wine derived from *Elaeis guineensis* as a component of an alternative culture medium for microbial growth

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Abstract

The high cost and limited accessibility of conventional culture media highlight the need for alternative formulations based on locally available, nutrient-rich materials, such as palm wine derived from *Elaeis guineensis* sap. This study aimed to develop and evaluate an alternative microbial culture medium containing palm wine derived from *E. guineensis* sap. The palm wine-based medium (PWM) was formulated using lyophilized palm sap supplemented with complementary nutrients to support microbial growth. Its performance was evaluated using 11 bacterial strains of food and public health relevance, 7 lactic acid bacteria, and 3 filamentous fungi, as well as microorganisms recovered from environmental samples collected from multiple surfaces. Microbial growth and recovery on PWM were compared with those obtained on plate count agar (PCA) for bacteria, de Man, Rogosa and Sharpe agar (MRS) for lactic acid bacteria, and potato dextrose agar (PDA) for filamentous fungi, according to the requirements of each microbial group. Analysis of variance was used to assess differences in microbial growth, while the two one-sided test procedure was used to assess equivalence within a predefined margin of ±0.5 $\log_{10}$ CFU mL^{-1}. Statistical equivalence was observed between PWM and PCA, as well as between PWM and MRS and between acidified PWM and PDA. These findings indicated that differences in microbial counts between PWM and the respective reference media remained within the predefined equivalence range, supporting the potential of PWM as an alternative to conventional culture media under the conditions evaluated. Its development contributes to the valorization of *E. guineensis* sap as a locally available biological resource, with potential applications in microbiological analysis and environmental monitoring, particularly in resource-limited settings.

Keywords: Alternative culture medium; Senegal; Bacterial growth; Formulation; Elaeis guineensis; Technological product

1. Introduction

Palm wine, derived from the sap of the African oil palm (*Elaeis guineensis*), is a traditionally fermented beverage obtained from palm sap, whose spontaneous fermentation is driven by diverse microbial communities and results in complex changes in its chemical composition.[1] This white, sweet-tasting sap undergoes rapid transformation: its alcohol content can increase to approximately 4% within hours of fermentation, and, if left untreated, it transitions to vinegar within a few days.[2] Palm wine contains sugars, proteins, amino acids, vitamins, and minerals that may contribute to microbial growth and therefore support its potential incorporation into culture medium formulations,[3] and the fermentation of palm sap is accompanied by significant changes in physicochemical and volatile constituents, reflecting the dynamic nature of this nutrient-rich plant-derived substrate.[4] The palm sap contains sugar, protein, fat, and mineral matter. It is generally regarded as safe and contains beneficial lactic acid bacteria (LAB) and Saccharomyces yeast species that possess probiotic attributes.[3] Furthermore, its acceptance extends even to individuals of Islamic faith, despite the religion's general prohibition against alcohol consumption, suggesting that the unfermented or minimally fermented forms are culturally and religiously acceptable.

In a recent study, Manicongo *et al.*[5] characterized palm wine derived from *E. guineensis* sap collected in the Casamance region of Senegal. A comprehensive chemical analysis was conducted, covering multiple parameters and applying advanced analytical techniques to ensure depth and accuracy. The palm wine had a pH of 4.16, a titratable acidity of 34 meq/L, and a total soluble solids content of 9.63 °Brix. Microbiological analysis confirms its safety, as no fecal coliforms, *Salmonella* spp., *Listeria monocytogenes*, or *Staphylococcus aureus* were detected. The initial yeast count was 2.1×10^2 colony-forming units (CFU)/mL. These physicochemical and microbiological characteristics indicate that palm wine is a nutrient-containing biological matrix that may contribute to microbial growth. Since sap is widely referred to as palm wine by local communities, whether freshly collected or fermented, the term "palm wine" is used throughout this study to refer to both forms, in accordance with local terminology.

The nutritional characteristics of palm wine provide a basis for investigating its incorporation as a biological component of an alternative microbiological culture medium. Culture media provide the nutrients and physicochemical conditions required for the growth and enumeration of microorganisms in vitro, including sources of carbon and nitrogen, vitamins, minerals, water, and, in solid media, a solidifying agent such as agar.[6] However, nutritional and physicochemical requirements vary among microorganisms. Therefore, the suitability of an alternative culture medium should be evaluated using different microbial groups and compared with conventional reference media appropriate for each group.

For bacterial enumeration, plate count agar (PCA) is widely used as a general-purpose medium for the recovery and enumeration of aerobic mesophilic bacteria. PCA typically contains 5.0 g/L of peptone, 2.5 g/L of yeast extract, 1.0 g/L of glucose (dextrose), and 15.0 g/L of agar. The peptone provides peptides and amino acids as nitrogen and carbon sources, yeast extract supplies vitamins and growth factors, glucose provides a readily available energy source, and agar serves as the solidifying agent. The final pH of PCA is generally adjusted to approximately 7.0 ± 0.2, a condition suitable for the cultivation and enumeration of a broad range of bacteria.[7]

For the cultivation of LAB, de Man, Rogosa and Sharpe agar (MRS) is one of the most widely used conventional culture media. MRS is a nutritionally rich medium containing carbon and nitrogen sources, vitamins, minerals, and other growth-promoting compounds required by LAB. Recent evidence further indicates that agricultural waste can serve as a low-cost alternative medium for the cultivation of LAB, supporting their potential application in resource-efficient microbiological processes.[8] Its composition typically includes glucose, peptones, yeast and meat extracts, mineral salts, and selective components that favor the growth of LAB. The medium generally has a slightly acidic pH, around 6.2, which further supports the cultivation of this microbial group.[9]

For the cultivation and enumeration of filamentous fungi and yeast, potato dextrose agar (PDA) is a commonly used reference medium. PDA is prepared from potato infusion, typically equivalent to 200 g of potatoes per liter, supplemented with 20 g/L dextrose and approximately 15 g/L agar. The potato infusion provides a complex source of nutrients, while dextrose serves as an assimilable carbon and energy source; agar provides the solid matrix required for microbial growth. In contrast to PCA, PDA is generally adjusted to an acidic final pH of approximately 5.6 ± 0.2, which favors the growth of fungi while reducing proliferation of many bacteria. Therefore, PCA, MRS, and PDA were considered distinct reference media, with PCA used for the cultivation and enumeration of general bacteria, MRS for LAB, and PDA for fungi. This distinction was particularly relevant to the present study because the palm wine-based medium (PWM) was evaluated against each reference medium according to the microbial group

investigated. Accordingly, PCA, MRS, and PDA served as reference media for the evaluation of bacteria, LAB, and fungi, respectively.

The valorization of agro-industrial residues as alternative microbial substrates has gained increasing attention as a strategy to improve the economic and environmental sustainability of biotechnological processes,[10] and recent studies have demonstrated the feasibility of formulating microbial culture media from agro-industrial by-products, offering low-cost alternatives to conventional commercial formulations.[11] Moreover, the conversion of food-processing residues into cultivation substrates has been proposed as a strategy for nutrient recovery, waste valorization, and the development of more sustainable biotechnological processes.[12] Such an approach may contribute to the development of a novel biotechnological product based on an organic and potentially sustainable substrate. Additionally, agro-industrial biowastes have been explored as alternative substrates for microbial growth and fermentation, contributing simultaneously to waste valorization and the reduction of substrate costs.[13] Systematic evidence also supports the use of alternative substrates and media as a means of reducing production costs while improving the sustainability of microbial bioprocesses.[14] The use of plant-derived substrates as alternatives to conventional culture media has gained increasing interest because of their nutritional composition, availability, and potential to reduce production costs.[15] A recent study demonstrated that palm wine contains sugars, organic acids, minerals, amino compounds, vitamins, and other bioactive constituents, supporting its potential as a nutrient-rich substrate for microbial cultivation.[5]

Previous studies have reported the use of plant-derived substrates and food-processing residues as alternative components of culture media, with several formulations supporting satisfactory microbial growth while potentially reducing production costs. According to Santos *et al.*,[15] plant-based materials provide essential nutrients, such as carbohydrates, proteins, vitamins, minerals, and growth-promoting compounds, making them suitable substrates for microbial cultivation while reducing dependence on commercially manufactured media. Given the specific nutritional requirements of LAB, the development of culture media based on alternative and nutrient-rich substrates has also received increasing attention.[9,16] Considering that palm wine contains fermentable sugars, amino compounds, vitamins, minerals, and organic acids, it may also provide nutritional conditions suitable for LAB cultivation. Therefore, evaluating its performance with LAB is important for determining the broader applicability of the proposed culture medium. Waste whey and whey-derived substrates have also been investigated as nutrient sources for microbial processes, including the microbiological production of lactic acid, highlighting their potential as alternative culture-medium components.[17]

Although palm wine itself contains several nutrients required for microbial growth, its natural composition may vary and may not provide all nutritional requirements in balanced proportions. Therefore, the test medium was formulated by supplementing palm wine with complementary nutrient sources. Sweet potato extract was included as an additional carbon source, providing complex carbohydrates; whey protein provided an additional source of nitrogen and protein-derived nutrients[18]; yeast extract supplied growth factors and B-complex vitamins; and agar was used as a solidifying agent. This multicomponent formulation was intended to provide nutrient sources comparable in function to those present in conventional culture media while maintaining a sustainable and locally sourced basis.

The suitability of the test medium was therefore evaluated using microorganisms representing different physiological and nutritional requirements. The study included pathogenic bacteria of food and clinical relevance, LAB, and fungi. For fungal assays, both the PDA reference medium and the test medium were acidified to restrict bacterial growth and provide conditions favorable for fungal development. The performance of the test medium was subsequently compared with PCA, MRS, and PDA, according to the microbial group evaluated.

Although our previous study focused exclusively on the physicochemical and microbiological characterization of palm wine as a traditional beverage, the present work introduces an independent technological application through the formulation, optimization, and validation of a novel microbiological culture medium. The composition of the test medium was rationally designed based on the nutritional requirements of microorganisms and the intrinsic biochemical properties of palm wine. Palm wine is a complex biological matrix rich in readily fermentable sugars, organic acids, amino compounds, minerals, and vitamins,[5] which are essential for microbial metabolism. However, as a natural substrate, it may present compositional variability and potential nutritional imbalances. Therefore, it was combined with complementary components to ensure a balanced and reproducible formulation. Sweet potato extract was included as an additional carbon source, providing complex carbohydrates; whey protein contributed a rich nitrogen source and essential amino acids[18]; yeast extract supplied growth factors and B-complex vitamins; and agar was used as a solidifying agent. This multicomponent design aims to mimic the

functional roles of conventional industrial media while maintaining a sustainable and locally sourced basis.

Therefore, the aim of this study was to evaluate the performance of a PWM as an alternative culture medium by comparing its ability to support the growth of food- and clinically relevant pathogenic bacteria, LAB, and fungi with that of conventional reference media, namely PCA, MRS, and PDA, respectively. The hypothesis was that a standardized PWM, supplemented with complementary nutrient sources and adjusted to an appropriate pH, would provide sufficient nutritional conditions to support the growth of bacteria and fungi at levels comparable to those achieved with conventional culture media.

2. Materials and methods

2.1. Formulation of the culture medium

To obtain the palm wine dry extract, the raw sap was subjected to ultrahigh-temperature treatment (140 °C for 4 s), followed by freeze-drying. The resulting lyophilized palm wine extract constituted the biological component under investigation and was incorporated into a standardized multicomponent culture medium formulation. The remaining ingredients, which are commonly used in microbiological media, were commercially sourced and included to provide complementary nutritional functions and ensure a balanced and reproducible formulation. The final culture medium, designated PWM, consisted of 45% (w/w) water-soluble sweet potato extract, 25% (w/w) lyophilized palm wine extract, 25% (w/w) skimmed whey protein, 3.5% (w/w) yeast extract, and 1.5% (w/w) agar. The reported percentages refer to the dry-weight composition of the formulation before hydration. Sweet potato extract served as an additional carbon source, whey protein supplied nitrogen and essential amino acids, yeast extract provided vitamins and growth factors, while agar functioned as the solidifying agent. In this formulation, palm wine was evaluated as a nutrient-rich biological component within a complementary multi-ingredient system rather than as the sole nutritional source. Proximate analysis of the PWM formulation yielded 46.58% protein, 44.62% total sugars, 5.34% ash, 2.03% dietary fiber, and 1.96% lipid, indicating a balanced nutrient composition suitable for microbial cultivation. PCA (Himedia, India) was used as the reference medium for bacterial growth comparisons and prepared according to the manufacturer's instructions. The final pH was 7.0 ± 0.2 at 25 °C. The PWM was similarly adjusted to pH 7.0 ± 0.2 before autoclaving using 0.1 M NaOH.

A preliminary assay was conducted using the PWM complete medium (45% [w/w] water-soluble sweet potato extract, 25% [w/w] lyophilized palm wine extract, 25% [w/w] protein fraction composed of skimmed whey, 3.5% [w/w] yeast extract, and 1.5% [w/w] agar-agar), commercial PCA, and a formulation containing all components except the lyophilized palm wine extract. *Escherichia coli* was used as the test microorganism. The results showed that the PWM complete medium and PCA yielded comparable counts, both detecting an average population of 9.3 $\log_{10}$ CFU/mL. In contrast, the medium lacking the lyophilized palm wine extract exhibited lower and more variable counts, ranging from 7.7 to 8.4 $\log_{10}$ CFU/mL. This outcome was expected, since PWM contains considerable amounts of fermentable sugars, amino acids, and minerals[3] that may serve as supplementary nutrients in the culture medium. Under these experimental conditions, the omission assay indicated that the inclusion of palm wine contributed to the performance of the complete formulation for *E. coli*. However, because this experiment was performed only with *E. coli*, the specific contribution of palm wine to the growth of the other microorganisms evaluated cannot be established.

Twenty-five grams of PWM culture medium were suspended in 1 L of distilled water. The pH was then measured and adjusted to 7.0 ± 0.2 by gradually adding 0.1 M NaOH or 0.1 M HCl solution, as necessary. Subsequently, the culture medium was sterilized by autoclaving at 121 °C for 15 min. Afterward, it was plated on sterile Petri dishes. To compare microbial multiplication in the reference medium, PCA was used, prepared according to the manufacturer's instructions, and also autoclaved at 121 °C for 15 min.

2.2. Microorganism strains

The microorganisms used in this study are listed in Table 1, together with their taxonomic classification and identification codes. The strains were obtained from the culture collections of the Laboratório de Microbiologia de Alimentos (DCTA/FAEM), the Laboratório de Processamento de Produtos de Origem Animal (DCTA/FAEM), and the Laboratório de Ciência e Microbiologia de Alimentos (CCQFA), all laboratories at the Federal University of Pelotas (UFPel), Brazil. The corresponding culture media and incubation conditions used for each microbial group are provided in the table note for Table 1.

2.3. Preparation of bacterial inocula

The reference microorganisms (*S. enterica* serovar Typhimurium, *A. hydrophila*, *E. coli*, *E. faecalis*, *L. monocytogenes*, *P. aeruginosa*, *P. fluorescens*, *S. aureus*, *S. epidermidis*, *S. mutans*, *S. typhimurium*, and *P. mirabilis*) were previously cultured, and the inoculum was standardized to a turbidity equivalent to 0.5 on the McFarland scale (~1.5 × 10^8 CFU/mL), as determined

Table 1. Classification of the microorganisms used in this study according to their taxonomic group

Classification	Microorganism	Identification	Source/reference
Bacterial strains relevant to food and public health	*Salmonella enterica* serovar Typhimurium	ATCC 14028	–
	E. coli	ATCC 25922	–
	S. aureus	ATCC 25923	–
	Pseudomonas aeruginosa	ATCC 15442	–
	L. monocytogenes	ATCC 7644	–
	Pseudomonas fluorescens	ATCC 13525	–
	Streptococcus mutans	ATCC 700610	–
	Staphylococcus epidermidis	ATCC 12228	–
	Aeromonas hydrophila	ATCC 11036	–
	Enterococcus faecalis	ATCC 29212	–
	Proteus mirabilis	ATCC 25933	–
Lactic acid bacteria	*Lactiplantibacillus plantarum*	CO5	–
	Leuconostoc mesenteroides	KLM6	19
	Pediococcus pentosaceus	KPP1	19
	Lacticaseibacillus casei	CSL3	20
	Enterococcus faecium	EO1	20
	Lactobacillus curvatus	P99	21
	Enterococcus thailandicus	F1	20
Filamentous fungi	*Aspergillus niger*	ATCC 9642	–
	Fusarium spp.	ATCC 22782	–
	Penicillium spp.	ATCC 36507	–

Notes: Reference bacterial strains were recovered from stock cultures on tryptic soy agar (Kasvi, Brazil) and incubated for 24 ± 2 h at 37 °C, except *P. aeruginosa* and *P. fluorescens*, which were incubated at 25 °C. Lactic acid bacteria were recovered from stock cultures using de Man, Rogosa, and Sharpe broth and incubated at 37 °C for 24 ± 2 h. Filamentous fungi were recovered from stock cultures on potato dextrose agar and incubated under the conditions recommended for each strain. Strains obtained from the American Type Culture Collection (ATCC) are identified by their unique accession numbers; therefore, separate bibliographic references are not provided. Bibliographic references are included for strains previously isolated and characterized in published studies. The dash (–) indicates that no bibliographic reference is provided.

spectrophotometrically at 600 nm (OD600 = 0.1) using a UV/VIS IL-592 spectrophotometer (Kasuaki, Japan). From this standardized suspension, serial tenfold dilutions were prepared in sterile saline solution, and 0.1 mL aliquots were inoculated onto the surface of PCA and PWM agar plates using the spread-plate technique. The plates were incubated at 37 °C for 24 ± 2 h under aerobic conditions. After the incubation period, CFUs were enumerated, and the results were expressed as $\log_{10}$ CFU/mL for comparison of microbial growth between the two culture media.

2.4. Lactic acid bacteria inocula

The LAB used in this study were previously characterized by the Laboratório de Processamento de Produtos de Origem Animal. The bacterial strains were reactivated prior to analysis, and the inoculum was standardized to a turbidity equivalent to the 0.5 McFarland standard (~1.5 × 10^8 CFU/mL), corresponding to an optical density at 600 nm (OD600) of 0.1. From the suspension, tenfold serial dilutions were prepared in tubes containing 0.9 mL of sterile saline solution. Subsequently, 0.1 mL aliquots of the appropriate dilutions were surface plated onto Petri dishes containing de Man, Rogosa and Sharpe (MRS) agar (Kasvi, Brazil) and PWM using the spread plate technique. The plates were incubated at 37 °C for 48 h ± 2 h under anaerobic conditions. After incubation, CFUs were enumerated, and the results were expressed as $\log_{10}$ CFU/mL to allow comparison of microbial growth between the two culture media.

2.5. Fungi inocula

Microbiological analyses were performed according to the recommendations of the American Public Health Association (APHA),[22] with the modifications described below. A standard fungal strain was inoculated onto PDA (Himedia, India) and incubated at 25 °C for 72 h to obtain young mycelium. A loopful of the fungal culture was transferred to a tube containing 10 mL of sterile saline solution to prepare the inoculum. When necessary, serial dilutions of the inoculum were prepared. The turbidity of the suspension was compared with the McFarland standard to estimate the microbial concentration of the inoculum. For the fungal assays, both the PDA and test medium were acidified prior to inoculation to inhibit bacterial growth and ensure selective conditions for fungal development. Subsequently, 100 µL aliquots of the saline suspension were spread onto the surface of acidified PDA and acidified test medium using the spread plate technique. The plates were incubated at 25 °C for 72 h, after which the colonies were counted. Each assay was performed in triplicate.

2.6. Environmental sampling

Samples were collected to evaluate and compare bacterial growth on two culture media, PWM and PCA. To this end, environmental samples were obtained from 12 different surfaces, including bathroom sink, cell phone, water tap, laboratory incubator, toilet, human hand, computer, sink sponge, microwave, mucosa, handrail, and currency. Surface sampling was performed using sterile swabs previously moistened with 0.1% peptone solution, which were rubbed across a delimited area of 10 cm^2 to ensure consistent sample recovery. Each surface was sampled in duplicate, and the collected material was immediately inoculated onto sterile Petri dishes containing PWM agar and PCA agar. The plates were incubated at 37 °C for 24 ± 2 h under aerobic conditions.

2.7. Statistical analysis

Statistical analyses were performed to compare microbial counts obtained on PWM and the corresponding reference media. Overall differences between culture media were evaluated using analysis of variance (ANOVA). In addition, equivalence was assessed separately for each microorganism using a paired two one-sided tests (TOST) procedure based on the paired $\log_{10}$ CFU/mL values obtained for PWM and the corresponding reference medium. The paired difference was calculated as PWM minus reference medium. An equivalence margin of ± 0.5$\log_{10}$ CFU/mL was defined *a priori* based on the criterion used for microbiological enumeration methods.[23] At a significance level of $\alpha = 0.05$, equivalence was established when the 90% confidence interval (CI) of the paired mean difference was entirely contained within the predefined equivalence bounds (−0.5 to +0.5 $\log_{10}$ CFU/mL). Environmental samples were evaluated descriptively using the semi-quantitative growth scale and were not included in the TOST equivalence analysis.

3. Results

An initial comparative analysis was performed between PWM and the reference culture media to evaluate the growth of microorganisms relevant to food and public health (Table 2). ANOVA revealed a statistically significant overall difference in microbial counts between PWM and PCA ($p = 0.0337$), whereas no significant differences were observed between PWM and MRS ($p = 0.0510$) or between acidified PWM and PDA ($p = 0.3162$). To further assess comparability, microorganism-specific equivalence was evaluated using the TOST procedure with a predefined margin of ±0.5 $\log_{10}$ CFU mL^{-1}. For all microorganisms evaluated, the 90% confidence intervals of the paired mean differences fell entirely within the equivalence bounds, supporting equivalence between PWM and the corresponding reference media under the experimental conditions. These results indicate that, despite the significant overall difference detected between PWM and PCA by ANOVA, the differences for individual microorganisms remained within the predefined microbiological equivalence margin.

In addition, PWM and PCA culture media were inoculated in parallel with environmental samples obtained from 12 distinct surfaces. Microbial recovery was evaluated semi-quantitatively using an ordinal scale: 0 = no growth; 1 = scarce growth; 2 = moderate growth; 3 = abundant growth. Presence or absence of microbial growth was concordant between PWM and PCA in all 12 samples (12/12, 100%). Eleven samples showed microbial growth in both media, whereas one sample showed no growth on either medium. Because quantitative colony counts were not obtained for this experiment, these environmental results should be interpreted as an exploratory semi-quantitative comparison and not as evidence of statistical equivalence between PWM and PCA.

Regarding growth intensity, three samples (25%) exhibited abundant growth, corresponding to plates 1, 8, and 9 (Figure 1). In four samples (33.3%), growth was classified as moderate, observed in plates 2, 5, 7, and 12. An equivalent number of samples demonstrated scarce growth (plates 4, 6, 10, and 11), whereas only one sample (8.3%), illustrated in plate 3, showed no microbial multiplication in either of the culture media.

Overall, the results demonstrated that PWM exhibited performance equivalent to PCA for all microbial groups

Table 2. Comparison of microbial counts between the PWM and conventional RM for bacterial strains, filamentous fungi, and lactic acid bacteria

Microorganism	PWM (mean $\log_{10}$ CFU/mL)	RM (mean $\log_{10}$ CFU/mL)	Mean difference	90% CI	TOST *p*-value	Equivalence conclusion
Bacteria						
Salmonella Typhimurium	9.45073	9.45516	−0.00443	−0.28963 to 0.28076	0.01836	Equivalence
E. coli	9.45008	9.43075	0.01933	−0.15352 to 0.19218	0.00742	Equivalence
S. aureus	9.39584	9.50373	−0.10789	−0.28090 to 0.06512	0.01104	Equivalence
L. monocytogenes	9.02273	9.09668	−0.07395	−0.41214 to 0.26424	0.03330	Equivalence
P. aeruginosa	10.1040	10.0852	0.01877	−0.22235 to 0.25990	0.01410	Equivalence
A. hydrophila	6.64334	6.74693	−0.10359	−0.13532 to −0.07185	0.00038	Equivalence
S. epidermidis	6.77728	6.85411	−0.07683	−0.48644 to 0.33278	0.04728	Equivalence
S. mutans	6.11148	6.49322	−0.38173	−0.46324 to −0.30022	0.02572	Equivalence
E. faecalis	7.92060	8.06339	−0.14279	−0.47436 to 0.18843	0.04393	Equivalence
P. fluorescens	7.89704	7.83570	0.06134	0.00570 to 0.11698	0.00094	Equivalence
P. mirabilis	7.72066	7.80851	−0.08785	−0.12503 to −0.05067	0.00048	Equivalence
Filamentous fungi						
A. niger	6.14697	6.15537	−0.00841	−0.11218 to 0.09537	0.00259	Equivalence
Fusarium spp.	6.40123	6.39845	0.00278	−0.10154 to 0.10710	0.00256	Equivalence
Penicillium spp.	6.47518	6.49004	−0.01486	−0.05825 to 0.02853	0.00047	Equivalence
Lactic acid bacteria						
P99	7.41431	7.57732	−0.16301	−0.46232 to 0.13631	0.04069	Equivalence
CO5	7.60161	7.64299	−0.04138	−0.07764 to −0.00513	0.00037	Equivalence
EO1	7.55596	7.63974	−0.08378	−0.17120 to 0.00363	0.00257	Equivalence
KPP1	7.40310	7.50878	−0.10568	−0.30697 to 0.09561	0.01461	Equivalence
KLM6	7.62292	7.72749	−0.10457	−0.22257 to 0.01343	0.00514	Equivalence
CSL3	7.75451	7.70384	0.05067	−0.03726 to 0.13861	0.00223	Equivalence
F1	7.56194	7.57110	−0.00916	−0.38145 to 0.36314	0.03067	Equivalence

Notes: Mean difference was calculated as PWM−RM. Equivalence was concluded when the 90% CI of the paired mean difference was entirely contained within −0.5 to +0.5 $\log_{10}$ CFU/mL.
Abbreviations: CI: Confidence interval; RM: Reference medium; TOST: Two one-sided tests; PWM: Palm wine-based medium.

evaluated, including pathogenic bacteria, filamentous fungi, and LAB. These findings support the broad applicability of PWM as a sustainable alternative culture medium capable of supporting the growth of diverse microorganisms.

4. Discussion

The results obtained in this study demonstrated remarkable consistency between the PCA and PWM culture media, indicating equivalent performance in recovering the microorganisms evaluated. The quantitative experiments demonstrated that PWM supported microbial growth within the predefined equivalence margin relative to the corresponding conventional reference media for the bacterial, fungal, and LAB strains evaluated. In contrast, the environmental sampling assay provided complementary exploratory evidence, showing concordant presence/absence of microbial growth between PWM and PCA across all 12 sampled surfaces. Because this environmental comparison was based on a semi-quantitative ordinal assessment rather than quantitative colony counts, it should not be interpreted as a formal demonstration of equivalence.

The statistically significant overall difference detected by ANOVA between PWM and PCA should be interpreted together with the microorganism-specific TOST

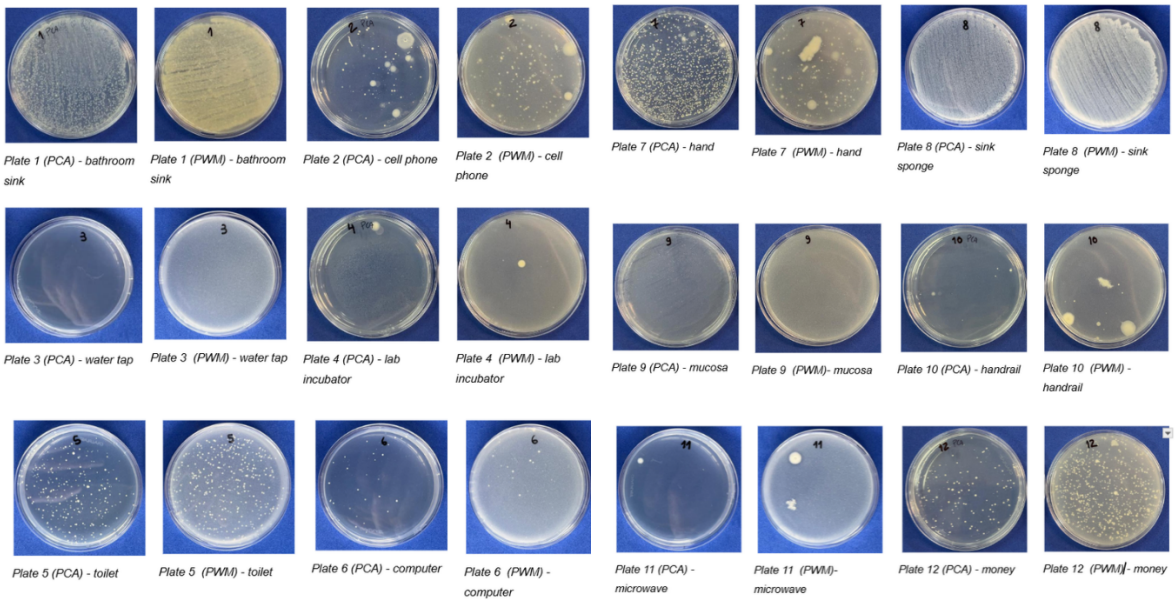


Figure 1. Comparison of palm wine-based medium (PWM) and plate count agar (PCA) culture media from samples collected from 12 different surfaces

results. ANOVA tests whether a statistically detectable overall difference exists, whereas TOST evaluates whether the magnitude of the paired difference for each microorganism remains within the predefined equivalence limits. Although the overall PWM–PCA ANOVA was significant (p = 0.0337), all microorganism-specific 90% CIs were contained within the prespecified −0.5 to +0.5 $\log_{10}$ CFU/mL bounds. Therefore, the two findings are not contradictory: a statistically detectable overall difference can coexist with microorganism-specific differences that remain within the predefined equivalence margin. These present findings are consistent with a recent study demonstrating the potential of media from agro-industrial by-products; Tchabou *et al.*[24] developed a medium based on pineapple peel, sugarcane molasses, and black soldier fly larvae cake and reported LAB growth ranging from 9.43 to 9.86 $\log_{10}$ CFU/mL, which was comparable to or higher than that obtained with conventional MRS and M17 media. These findings support the concept that combinations of nutritionally complementary agro-industrial substrates can provide suitable sources of carbon, nitrogen, minerals, and growth factors for microbial cultivation. Similarly, the present study demonstrates that the PWM formulation can support the growth of different microbial groups, although its performance varied according to the microorganism and the conventional medium used for comparison

Similar findings have been reported by Shareef.[25] who compared total count media and reinforced the robustness of PCA for assessing microbial load, while highlighting the potential of alternative media as sustainable and cost-effective options. In this context, PWM may represent a viable alternative, particularly in resource-constrained laboratories or regions seeking to utilize locally available agricultural resources. Recent studies have shown that plant-based and low-cost culture media formulated from soybean,[26] cereals such as chickpea and rice,[25] and agro-industrial residues[27] can effectively support microbial growth with performance comparable to commercial media. In general, vegetable waste has also been evaluated as alternative nutrient sources for fungal cultivation, with several fungal species showing substantial growth on waste-derived media compared with conventional formulations.[28]

The present study demonstrated that the complete PWM formulation supported the growth of pathogenic bacteria, filamentous fungi, and LAB at levels equivalent to those obtained with the respective reference media. Because PWM contains palm wine together with sweet potato extract, whey protein, yeast extract, and agar, this performance should be attributed to the combined nutritional contribution of the formulation rather than to palm wine alone.

Similarly, the ability of PWM to support the growth of LAB broadens its potential applications. LAB are nutritionally demanding microorganisms requiring fermentable carbohydrates, amino acids, peptides, vitamins, and minerals for optimal growth.[9] The equivalence observed in this study indicates that PWM

provides an adequate nutritional environment for these microorganisms and may represent a sustainable alternative for their cultivation. This finding is particularly relevant considering the increasing interest in optimizing cultivation media for LAB to improve their technological and biotechnological applications, including the production of metabolites such as exopolysaccharides.[19]

Although alternative plant-based media have demonstrated considerable potential, they often present limitations related to natural variability in substrate composition, which may affect reproducibility and microbial performance. Responses may vary according to the microbial species, and differences in nutrient composition may require adjustments in pH, extract concentration, or nutrient supplementation. In the present study, these limitations were minimized through the standardization of palm wine processing, including controlled collection, thermal treatment, and freeze-drying prior to medium preparation. Once standardized, PWM consistently produced results equivalent to those obtained with the commercial reference medium, supporting its potential as a sustainable and reliable alternative for microbiological applications.

Future studies should address practical limitations of PWM, including its opacity, which may interfere with colony visualization and enumeration, as clear observation, differentiation, and enumeration of microorganisms are essential for the accurate detection of pathogens. These factors may otherwise compromise the reliability of visual assessments. Despite such limitations, the development of optimized formulations and standardization protocols could further enhance the performance of alternative media, making them viable options for environmental and educational microbiological applications. This study is fully aligned with Sustainable Development Goals 2, 3, and 12, contributing to advances in food safety, public health, and sustainable production practices.

The relatively high protein content of PWM compared with conventional PCA represents an important compositional difference that could influence microbial growth. However, no inhibitory or selective effects were observed under the experimental conditions. Instead, PWM supported equivalent growth of pathogenic bacteria, filamentous fungi, and LAB, indicating that its nutritional composition provides adequate carbon, nitrogen, and growth factors for microorganisms with distinct physiological requirements. The successful cultivation of filamentous fungi is particularly relevant, as PDA is the standard medium recommended for fungal cultivation.[22] Likewise, the equivalent growth of LAB demonstrates that PWM provides nutrients compatible with the complex nutritional requirements of these microorganisms, including carbohydrates, amino acids, vitamins, and minerals.[9] Overall, these findings indicated that, despite its distinct nutritional profile, PWM supported the growth of all microbial groups evaluated under the tested conditions.

Although the present study does not aim to introduce a new microbiological concept or replace standardized culture media for diagnostic purposes, its scientific contribution lies in the rational development, validation, and standardization of a culturally rooted, technological product for microbiological applications. Unlike many previously reported plant-based media, which rely on single raw substrates or non-standardized extracts, the PWM medium was formulated from multiple nutritionally complementary components and subjected to controlled processing steps (thermal treatment, lyophilization, proximate analysis, and pH standardization), thereby ensuring reproducibility and biosafety. The equivalence observed between PWM and PCA should therefore not be interpreted as merely confirmatory, but rather as a critical validation step required for any alternative medium intended for practical use, particularly in educational, environmental, and resource-limited laboratory settings. Also, the palm wine used in this study originated exclusively from Senegal, where harvesting procedures are well established and standardized. At present, standardized palm wine production is not available in Brazil. Ongoing research by our group aims to establish extraction protocols under Brazilian conditions. Consequently, the present work should be interpreted as a proof-of-concept based on a standardized Senegalese raw material. Although the nutritional composition of palm wine may vary according to palm species, geographical origin, harvesting season, and fermentation stage, the use of controlled collection procedures followed by thermal treatment and lyophilization minimized variability within the batch evaluated in this study. Future investigations should assess inter-batch variability and raw material obtained from different regions and palm species.

Moreover, the innovation of this work resides in the technological valorization of palm wine, a traditional African product, into a microbiological input with defined composition and performance. This approach integrates microbiology, food science, and potentially sustainable technology, contributing to the diversification of culture media sources and reinforcing the feasibility of locally derived laboratory consumables. While broader benchmarking and mechanistic studies remain necessary to expand its applications, the present results establish a robust proof-of-concept that supports future optimization, functional differentiation, and inter-laboratory validation.

5. Conclusion

This study developed and evaluated a culture medium (PWM) formulated using lyophilized palm wine from *E. guineensis* together with complementary nutritional and solidifying components. The medium was evaluated for its ability to support the growth of bacteria, LAB, and filamentous fungi and was compared with conventional culture media, including PCA, MRS, and PDA. Microbial growth was assessed using statistical and equivalence analyses. Under the conditions tested, PWM showed comparable microbial growth to the respective reference media, supporting its potential as an alternative culture medium formulation.

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Conflict of interest

We confirm that there are no personal relationships or conflicts of interest that could have influenced the research, including relationships with individuals or organizations mentioned in the paper.

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Ethics approval and consent to participate

The samples were previously collected as part of a research project approved by the Research Ethics Committee of the School of Medicine at the Federal University of Pelotas (UFPEL) (CAAE: 63622522.3.0000.5317). Informed consent was obtained from all participants before sample collection.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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