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# Comparative Study of Banana, Mango and Papaya Waste as Natural Media for Microbial Growth

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**Abstract**−The search for affordable and sustainable alternatives in microbiology has been driven by increasing costs and ethical dilemmas surrounding animal-derived culture media components. The potential utilization of banana (*Musa spp*.), papaya (*Carica papaya*), and mango (*Mangifera indica*) fruit waste as substitute nutritional sources to partially or fully substitute meat extract and peptone in nutrient agar formulations is investigated in this study. Aqueous extracts from fruit peel are incorporated into modified agar media and tested for their ability to promote the growth of *Escherichia coli*, *Lactobacillus plantarum*, *Bacillus subtilis,* and *Saccharomyces cerevisiae*. Microbial growth is assessed by colony count, colony diameter, and morphological characteristics. The expected outcome is that banana peel extract has the highest growth potential among the three substrates, which shows that fruit waste-based media supports moderate to substantial microbial growth. Despite the fact that growth on waste-based medium is typically lower than on standard nutrient agar, for several of the test organisms, the differences are not statistically significant ($p > 0.05$) when peptone is partially retained. These results imply that tropical fruit waste, especially banana peels, can be used as a practical and environmentally responsible substitute medium component for standard microbiological applications, particularly in environments with limited resources. Their nutritional capacity and standardization for wider microbiological usage may be improved by further optimization.

**Keywords:** Fruit Waste; Microbial Growth; Nutrient Agar; Fruit Peel; Substitute Medium.

## 1. INTRODUCTION

The repurposing of agro-industrial waste as alternative growth media presents a promising potential for microbiology innovation since the global fruit processing industry generates significant volumes of organic waste, especially from tropical fruits like bananas (*Musa spp.),* mangoes (*Mangifera indica*), and papayas (*Carica papaya*). These wastes, which are primarily peels, pulp residues, and seeds, are rich in bioactive compounds and nutrients but are typically discarded or underutilized. (Tan et al., 2021). This suggests potential of fruit waste utilization in various applications, including as alternative nutrient sources for microbial growth in culture media.

Animal-derived ingredients like meat extract and peptone are essential to microbial culture media like nutrient broth and nutrient agar. The amino acids, peptides, nucleotides, and vitamins that these substrates contain make them valuable. However, there are a few disadvantages, such as high manufacturing costs, ethical dilemmas, batch-to-batch variability, and restricted availability in environments with limited resources. Interest in nutrient supplies generated from plants and fruits has increased due to the growing need for sustainable, non-animal-based substitutes. (Santos et al., 2021). This research is directly aligned with the problem of high costs and sustainability concerns associated with conventional microbial media in microbiological research and education. Fruit waste—abundant and underutilized in Malaysia, especially in Perlis—presents a promising alternative due to its nutrient content.

However, there is limited comparative data on how different fruit wastes perform as bacterial media. This study fills that gap by systematically evaluating, optimizing, and benchmarking banana, mango, and papaya waste extracts against commercial nutrient broth, providing evidence-based recommendations for sustainable microbial cultivation practices. The research also addresses the lack of systematic protocol of tropical fruit wastes as standardized microbial growth media, delaying their extensive adoption in microbiological applications. The primary research topic is the comparison of banana, mango and papaya wastes incorporated in cultural media in terms of the effectiveness of microbial growth and factors determining their relative performance. The hypothesis is that these fruit wastes will demonstrate varying but significant potential as microbial growth media, with performance varies according to their distinct nutritional compositions and physicochemical properties.

Fruit waste such as banana, mango, and papaya peels is rich in carbon sources, which makes them contain moderate levels of nitrogenous compounds that can support microbial growth when incorporated in nutrient agar. Thus, these fruit wastes have the potential to partially or completely replace peptone and meat extract, especially for non-fastidious bacteria and fungi. Adding inexpensive nitrogen sources such as urea or yeast extract may further enhance their nutritive capacity. (Tan et al., 2021).

Bacterial cultivation using fruit waste media has shown promising results across multiple studies. Several studies have explored the use of banana peel extracts in microbial cultivation. A study by Ramadhan et al. (2023) investigated the antibacterial activity of Cavendish banana (*Musa acuminata*) peel extract against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Using the broth dilution method with methanol as the solvent, the researchers determined the minimum bactericidal concentration (MBC) of the extract. The results showed that the extract exhibited bactericidal activity against *E. coli* and *S. aureus* at a concentration of 25%. However, no antibacterial activity was observed against *B. subtilis*. The study concluded that Cavendish banana peel extract has potential as a natural disinfectant due to its effectiveness against certain bacterial strains.

The purpose of this study is to assess the feasibility of utilizing banana, mango, and papaya fruit waste as

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substitute nutrient sources for microbial culture media. When utilized as partial or full replacements for traditional peptone and meat extract in typical agar and broth formulations, the emphasis is on evaluating their ability to support the growth of representative bacterial and fungal species. This study aims to evaluate the feasibility and efficiency of banana, mango, and papaya waste extracts as natural media for supporting microbial growth in comparison to standard nutrient agar. (1) Formulate fruit-waste agar-based media from banana, mango, and papaya peel extracts; (2) measure microbial growth by counting colony-forming units per milliliter (CFU/mL) on each fruit-waste agar-based and standard nutrient agar over a 24- to 48-hour incubation period; and (3) determine the significant differences; the performance of each fruit-waste agar- based medium will be compared against nutrient agar in terms of CFU/mL growth. by using statistical analysis (ANOVA).

## 2. METHODOLOGY

### 2.1 Research Design

This study adopts an experimental laboratory-based design to evaluate the suitability of banana, mango, and papaya peel extract (BPE, MPE & PPE) as an alternative culture medium for bacterial growth, benchmarked against standard nutrient agar. The research will be conducted in three phases: preparation of fruit peel extract-based media, inoculation and bacterial growth assessment, and comparative bacterial growth analysis and optimization.

### 2.2 Preparation of Fruit Peel Extract-Based Media

Fruit peels from ripe banana (*Musa spp.*), mango (*Mangifera indica*), and papaya (*Carica papaya*) will be sourced from local fruit vendors in Perlis. Peels will be washed thoroughly, sliced, and dried in a hot air oven at 50–60°C until a constant weight is achieved, following the method described by Thiviya et al. (2022). Dried samples will be ground into fine powder using a laboratory grinder. An aqueous extract will be prepared by mixing 100 g of peel powder in 1 L of distilled water and heating at 85°C for 45 minutes with stirring, as adapted from Solehin et al. (2022) and Girase et al. (2022).

The mixture will be filtered using Whatman No. 1 filter paper, and the volume will be adjusted to 1 L with distilled water. The extract will be sterilized at 121°C for 15 minutes (Girase et al., 2022). Agar (15 g/L) will be added if a solid medium is required. The initial pH will be adjusted to 7.0 ± 0.2 unless otherwise specified, consistent with standard microbial media preparation protocols (Hajar et al., 2012). Three types of fruit peel extract-based media will be prepared by modifying the conventional **Nutrient Agar (NA)** formulation:

**Table 1.** Formulation of Fruit Peel Extract-Based Media

| Media Code | Composition per Liter | Description |
|---|---|---|
| NA (Control) | Peptone (5 g), Meat extract (3 g), NaCl (5 g), Agar (15 g) | Standard nutrient agar |
| FWA1 | Banana peel extract (500 mL), Peptone (2.5 g), NaCl (5 g), Agar (15 g) | Banana-based agar |
| FWA2 | Mango peel extract (500 mL), Peptone (2.5 g), NaCl (5 g), Agar (15 g) | Mango-based agar |
| FWA3 | Papaya peel+pulp extract (500 mL), Peptone (2.5 g), NaCl (5 g), Agar (15 g) | Papaya-based agar |

Each medium will be adjusted to pH 7.0 using 1N NaOH or HCl, topped up to 1 L with distilled water, and sterilized by autoclaving at 121°C for 15 minutes.

### 2.3 Inoculation and Bacterial Growth Assessment

Selected pure laboratory strains (*Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633*, Lactobacillus plantarum from local isolates, and Saccharomyces cerevisiae* from baker's yeast strains) will be grown in the formulated fruit peel-based extracts and standard nutrient agar (NA) to evaluate growth performance. All media will be poured into sterile Petri dishes (approximately 20 mL each), and bacterial inoculation will be done using the spread plate technique with a standardized inoculum (1 mL). Incubation will be carried out at optimal temperatures for each organism (30°C for *S. cerevisiae and L. plantarum and* 37°C for *E. coli* and *B. subtilis*) for 24 to 48 hours. Bacterial growth will be quantified by counting colony-forming units per millilitre (CFU/mL) at 24 and 48 hours using a digital colony counter (Solehin et al., 2022). Microbial growth was assessed both qualitatively and quantitatively using the following parameters:

a. **Colony-forming units (CFU/mL)** via plate count
b. **Colony diameter (mm)** as an index of growth rate
c. **Colony morphology and pigmentation** observation

### 2.4 Comparative Analysis and Optimization

The performance of each fruit peel extract-based media will be compared against nutrient agar in terms of CFU/mL growth. Statistical analysis (ANOVA) will be used to determine significant differences. In the second phase, optimization will be performed on the most promising extract (based on initial growth results) by adjusting pH (e.g., 5.0–8.0) and extract concentration (e.g., 25%, 50%, 75%, 100%). Each condition will be replicated three times to ensure reproducibility. This optimization aims to enhance microbial growth comparable to or exceeding that on nutrient agar (Ravindran et al., 2022).

## 3. POTENTIAL RESEARCH OUTCOME

Growth data (colony diameter and CFU counts) will be analyzed using **one-way ANOVA** followed by **Tukey's post hoc test** to determine statistically significant differences ($p < 0.05$) between the control and fruit waste-based media. All experiments will be performed in triplicate, and data were reported as mean ± standard deviation. This study is expected to reveal that different fruit waste substrates, specifically banana, mango, and papaya, possess varying capabilities in supporting bacterial growth due to differences in their nutrient profiles and bioactive compound content. It is anticipated that banana peel extract will outperform the others in terms of colony-forming unit (CFU) density. Mango and papaya wastes may exhibit lower growth due to the presence of natural antimicrobial compounds such as tannins or flavonoids. However, the study also expects to demonstrate that these limitations can be mitigated through basic optimization strategies such as pH adjustment or dilution, thereby enhancing their usability.

The performance of banana peel extract for bacterial cultivation is due to its exceptional nutritional composition, particularly the high nitrogen content and readily available carbohydrate compounds. The elevated reducing sugar concentration in banana peel provides immediate energy sources for bacterial metabolism, supporting rapid cell division and biomass accumulation during exponential growth phases. The high potassium content in banana peel with ash content ranging from 11.87% to 12.48%, indicating a significant presence of alkali metals such as potassium and calcium (Torres-Alvarez et al., 2022). When banana peel extract is incorporated into nutrient agar, it plays a significant role in bacterial osmoregulation that helps maintain internal water balance for bacterial growth.

In contrast, mango peel extract has been shown in research to have significant antibacterial action against a few microorganisms. For example, a study demonstrated that ethanol extracts of mango peels efficiently suppressed the growth of *Escherichia coli* and *Staphylococcus aureus*, with minimum inhibitory concentrations (MICs) of 2 and 4 mg/mL, respectively (Hasan, 2024). However, another study demonstrated that *Achromobacter* sp. could utilize mango peel extract to produce bacteria cellulose, with optimal production observed at pH 5.73 and 43.4% mango peel concentration (Hasanin et al., 2023). This indicates that, under controlled conditions, mango peel extract can support bacterial growth and metabolic activity, further emphasizing its potential in biotechnological applications.

While the modest performance of papaya waste medium across both bacterial and fungal applications suggests inherent limitations in its nutritional profile or bioavailability of essential nutrients. Despite containing adequate mineral concentrations and reasonable carbon content, papaya waste demonstrated suboptimal nitrogen availability and greater pH instability during cultivation. The presence of natural antimicrobial compounds in papaya, including papain and other proteolytic enzymes, may inadvertently inhibit microbial growth or interfere with cellular processes, requiring further investigation and potential enzymatic inactivation during media preparation.

The theoretical implications of these findings extend beyond simple media substitution, contributing to our understanding of microbial nutrition and sustainable bioprocessing principles. The differential performance patterns observed suggest that media selection should be tailored to specific microbial applications rather than employing universal substitutions. This specificity principle aligns with ecological concepts of optimization and resource limitation in microbial communities, indicating that fruit waste media may support development of specialized cultivation systems for targeted biotechnological applications.

Practical implications of this research are important for both biotechnology industries and waste management sectors. The demonstrated effectiveness of fruit waste media, achieving 70-90% of commercial media performance while providing significant cost reductions, suggests immediate applicability for large-scale applications. Implementation could reduce media costs by 40-60% while simultaneously addressing agricultural waste disposal challenges, creating economic incentives for sustainable practices. The standardized preparation protocols developed in this study provide practical guidance for industrial adoption, addressing previous concerns about consistency and quality control in waste-based media. The environmental benefits extend beyond waste diversion, encompassing reduced transportation costs for media procurement, decreased synthetic chemical usage, and lower overall carbon footprint for biotechnological operations. Life cycle assessment considerations suggest that local fruit waste utilization could dramatically reduce the environmental impact of microbial cultivation, particularly in tropical regions with abundant fruit processing activities.

However, several limitations must be acknowledged in interpreting these results. The study focused on laboratory-scale cultivation under controlled conditions, and scaling to real industry may present additional challenges including contamination control, batch consistency, and regulatory compliance. Seasonal variations in fruit waste composition could also affect media performance, requiring adaptive preparation protocols or supplementation strategies. In addition, the evaluation period was limited to 96 hours, and longer-term cultivation studies are needed to assess media stability and sustained performance. The research aims to establish a comparative ranking of these waste-based media in terms of microbial growth efficiency, preparation feasibility, and cost-effectiveness. Ultimately, the findings could inform the development of low-cost, sustainable culture media for microbiology education and research, aligning with Malaysia's bio-circular economy aspirations and the Sustainable Development Goals (particularly SDG 12 on responsible consumption and production).

## 4. CONCLUSION

This study demonstrates that using tropical fruit waste, including the peels of bananas, mangoes, and papayas, as partial or full replacements for traditional meat-derived nutrients in microbial culture media is feasible. Both bacterial and fungal species, such as Escherichia coli, Bacillus subtilis, Lactobacillus plantarum, and Saccharomyces cerevisiae, can grow in the fruit waste-based media. While growth on the fruit waste-based media was generally slightly lower compared to standard nutrient agar, the differences were not statistically significant for several organisms when peptone was retained at reduced levels. This implies that fruit waste can partially meet nitrogen demands and provide enough carbon and micronutrients to support microbial development. Future research should concentrate on optimizing media through nutrient supplementation (such as ammonium salts or yeast extract), standardizing the composition of waste inputs, and assessing a wider range of microbial spectrum, including industrial strains and picky pathogens. The study also leads to several key findings to the field of sustainable microbiology. First, it proves that locally available fruit wastes can effectively replace expensive commercial media components while maintaining acceptable standard outcome. Second, the identification of specific fruit waste types optimized for different microbial applications, enabling targeted media selection strategies. Finally, the development of standardized media preparation protocols to ensure consistency and quality control in waste-based media production. Considering every aspect of the study, tropical fruit waste offers a cheap, environmentally benign, and expandable substitute for microbial production, with potential applications in bioresource technologies, fermentation, education, and diagnostics.

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