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Original Research Article

Isolation, Identification, and Management of Microorganisms Associated with the Spoilage of Ripe Mango Fruit in Dutse, Nigeria

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Abstract

This study examined the microbial spoilage of ripe mango fruit (*Mangifera indica* L.) harvested from Dutse of Jigawa State, Nigeria and the effectiveness of physical, chemical and biological post-harvest management practices. Samples of mango were taken from the local market and farms and analyzed in the lab. The results showed that higher incidence of fungal pathogens, Aspergillus niger (35%) and *Penicillium species* (30%), were observed followed by yeast (*Saccharomyces cerevisiae*, 24%) and *Pseudomonas aeruginosa* (11%). For all the microorganisms isolated, pathogenicity tests showed that the microorganisms had different virulence levels; the most virulent microorganism was A. niger. The use of refrigeration at 10°C was found to be most effective among the management practices tested, resulting in 90% reduction in microbial growth and 10 days shelf life of the fruit. The chemical treatment using 0.1% w/v sodium benzoate also proved to be very effective with a microbial reduction of 85% for a shelf-life extension of 7 days. After 5 days, Modified Atmospheric Packaging (MAP) decreased microbial growth by 80% with an extension of 8 days whereas a dip in 1% (w/v) calcium chloride decreased microbial growth by 75% with an extension of 5 days. Biological agent *Bacillus subtilis* was effective in suppressing microbial growth by 70% and prolonging shelf life by 6 days, which was considered as a bio-control agent. A significant difference ($P < 0.05$) was observed between the different management strategies in terms of microbial growth reduction. The results of this study reveal that microbial spoilage is a major problem in mango production in the Dutse region and the series of effective and practical post harvest interventions recommended here can help reduce these losses and enhance food security and livelihood outcomes for farmers in the region.

Introduction

Mangifera indica L., a climacteric fruit is of tremendous economic and social importance worldwide. It is grown in more than 85 countries and a staple of tropical and subtropical agriculture (Lebaka et al., 2021). Asia is the world's largest producer accounting for around 77% of the world's production, followed by Africa with about 9%. Nigeria is one of the largest producers of cassava in Africa, ranking the highest in West Africa and amongst the world's largest producers with an estimated production of 0.8 million metric tons annually (Lebaka et al., 2021; Ogunbode et al., 2024).

Mango production is an important source of livelihood for millions of farmers in Nigeria, especially in the rural areas, and a major source of export earnings and gross domestic product for the country. With the mango market expected to grow into a $89.55 billion industry by 2028 (Tendata, 2024), it's clear that the mango industry has significant potential to contribute to foreign exchange generation. However, a pervasive problem that is of critical concern is the widespread post-harvest losses (Liu et al., 2022).

The main causes of such losses are related to the inherent short storage life of the mango fruit and are compounded by the lack of adequate post-harvest handling practices, lack of modern preservation techniques and infrastructure. Indigenous techniques such as sun-drying or drying in huts are traditional and less effective methods of preservation for most farmers and only provide some protection against spoilage. This causes substantial economic damages, food waste and a larger environmental impact (Moreno-Hernandez et al., 2024).

To overcome this, a local, scientific solution is needed to determine the microflora of a particular area and assess feasible, effective and sustainable control mechanisms. The ripe mango fruit in Dutse, Jigawa State is an interesting crop because of the climate condition that favours the growth of the crop and challenges in the preservation of the fruit. This study can contribute to unlocking this economic potential with a view of export promotion and diversification of income sources for farmers. This study is aimed at isolating, identifying and characterizing the microorganisms isolated from the ripe mango fruit that cause spoilage and examination of the effectiveness of the selected physical, chemical and biological treatment methods for controlling the microorganism causing the spoilage of the ripe mango fruit in Dutse of Jigawa state.

Materials and methods

Sample Collection and Processing

The ripe mango fruits were harvested from local market and farms in Dutse, Jigawa State, Nigeria. Fruits with mild to moderate decay symptoms were chosen to ensure good sampling of spoilage; more severely decomposed fruits might have harbored a variety of secondary, non-causal microorganisms. The fruits were carefully handled to prevent further mechanical damage and after that, fruits were transferred to sterile polythene bags and transported to Laboratory in a cool container for maintaining their physiological state and to prevent them from further microbial proliferation (Tam & Huyen 2024).

Isolation and Cultivation of Microorganisms

For the isolation of fungi, Potato Dextrose Agar (PDA) was used. The preferred nutrient base for potato infusion, PDA is a general purpose medium for the culture and identification of fungi, containing a dextrose source of carbohydrate for luxuriant growth and sporulation. Dehydrated PDA was suspended in 1 liter of distilled water and heated to boiling in the medium and then autoclaved for 15 minutes at 121°C. The pH of the medium was adjusted to about 5.6. The flesh of mangoes were cut and placed in PDA and left to stand at 30 °C for 5 – 7 days (Tam & Huyen 2024). Nutrient Agar was used for the isolation of bacteria. The nutrient agar was prepared by mixing the ingredients and autoclaving at 121°C for 15 minutes and the final pH was adjusted to 6.8.

Morphological Identification

Macroscopic morphological traits observed on the Agars were used as a first step to differentiate fungal and bacterial colonies. Fungi produce hyphae which are filamentous and form extensive mats, hence fungi could be distinguished from other fungi colonies by their characteristic powdery, hairy or filamentous appearance (Tam & Huyen 2024). Bacterial colonies, on the other hand, came up as minute (oily) spots that were smooth, moist or glistening. The color and texture of the colonies along with the shape and margin were also employed for differentiation. The isolated fungi were further examined under the microscope to observe their mycelium and conidiophores in order to achieve a proper identification of the fungi (Cheesebrough 2006).

Pathogenicity Test Protocol

To determine the virulence of each isolated microorganism and to validate their role as causal agents of mango spoilage by following Koch's postulates, a pathogenicity test was performed. The mangoes were chosen for the assay from the disease-free section of the orchard, where they had a uniform size and color (Tam & Huyen 2024). The fruits were surface disinfected using 75% alcohol. Each fruit was prepared with a standardized wound by sterilized needle-stabbing to promote infection. Few cells of each isolated microorganism were prepared in the form of spore or cell suspension and 5 μL was applied to the wounds by using pipettes. The sterile distilled water was used as the control inoculant for fruits. Inoculated fruits were then transferred to a humid condition, with relative humidity of about 95% and temperature of 25°C, for the growth of the pathogen. The severity of the disease was based on scores of 1 to 5, where 1 was no visible decay and 5 was the most severe disease symptoms. The progression of the disease was quantified by measuring lesion diameters and other symptoms daily (Chen et al., 2017).

Evaluation of Management Strategies

The effectiveness of the different management strategies was assessed by treating healthy ripen mangoes and assessing their microbial growth and shelf life.

Physical Treatments: Mangoes were refrigerated under controlled condition with temperature of 10°C or 25°C (Patil et al., 2019). In the case of Modified Atmospheric Packaging, fruits were packaged in bags containing modified atmosphere (Ramayya et al., 2012).

Chemical Treatments: Fruits were dipped for 5 minutes in either a 0.1% w/v solution of sodium benzoate or a 1% w/v solution of calcium chloride. The fruits were dipped, air dried and stored (Kaur et al., 2019).

Biological Treatment: A suspension of *Bacillus subtilis* was used to treat mangoes (Rivera-Chaves et al., 2020).

All treated fruits, along with an untreated control group, were stored under ambient conditions (unless specified otherwise for physical treatments). The effectiveness of each treatment was evaluated in terms of the percentage decrease in microbial count and number of days the shelf life was extended from the control treatment.

Data Analysis

All data, which was obtained from experiments in the work and was quantitative, have been analyzed statistically. The data were analysed using Analysis of Variance (ANOVA) at $P < 0.05$ level of significance to test for any statistically significant differences between the different treatments.

Results and Discussions

Results

Identification and Prevalence of Microorganisms

The analysis of microbial isolates from the spoiled mango fruits revealed a diverse microbial community, with a clear dominance of fungal species as shown in table 1. Four distinct microorganisms were isolated and identified based on their morphological characteristics and pathogenicity.

Table 1: Microbial isolates of spoiled mango

Organism	Classification	Percentage of Total Isolates
A. niger	Fungus	35%
P. species	Fungus	30%
S. cerevisiae	Yeast	24%
P. aeruginosa	Bacterium	11%

The most prevalent microorganism was the fungus *Aspergillus niger*, which accounted for 35% of all isolates. This was followed closely by another fungal species, *Penicillium* species, which comprised 30% of the isolates. The yeast *Saccharomyces cerevisiae* was the third most prevalent organism at 24%, while the bacteria *Pseudomonas aeruginosa* accounted for 11% of the isolates.

Pathogenicity of Isolated Microorganisms

Pathogenicity tests confirmed the virulence of all four isolated microorganisms, though their capacity to cause spoilage varied significantly. The severity of the symptoms was rated on a scale of 1 to 5, where 5 indicated the most severe spoilage as represented in table 2.

Table 2: Pathogenicity of Isolated Microorganisms

Organism	Pathogenicity Score (1-5 Scale)
A. niger	5
P. species	4
S. cerevisiae	3
P. aeruginosa	3

Aspergillus niger caused the most severe symptoms, earning a score of 5, indicating its high virulence. *Penicillium* species demonstrated significant pathogenicity with a score of 4. *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* showed a moderate level of virulence, each receiving a score of 3.

Efficacy of Management Strategies

The application of various physical, chemical, and biological treatments had a measurable impact on the post-harvest quality of the mangoes as shown in table 3. Each strategy showed a distinct level of effectiveness in reducing microbial growth and extending the shelf life of the fruit.

Refrigeration at 10°C was the most effective single treatment, reducing microbial growth by an impressive 90% and extending the shelf life of the fruit by 10 days. The chemical dip in a 0.1% w/v sodium benzoate solution was the next most effective treatment, reducing growth by 85% and extending shelf life by 7 days. Modified Atmospheric Packaging also demonstrated high efficacy, with an 80% reduction in microbial growth and an 8-day shelf life extension. The 1% w/v calcium chloride dip reduced microbial growth by 75% and increased shelf life by 5 days. Finally, the biological treatment with *Bacillus subtilis* was also effective, reducing microbial growth by 70% and extending shelf life by 6 days. A one-way ANOVA revealed a statistically significant difference in microbial growth reduction among the different management strategies ($P < 0.05$).

Table 3: Efficacy of Management Strategies

Treatment	Microbial Growth Reduction	Shelf Life Extension
Refrigeration (10°C)	90%[a]	10 days
Sodium Benzoate (0.1% w/v)	85%[b]	7 days
Modified Atmospheric Packaging (MAP)	80%[c]	8 days
Calcium Chloride (1% w/v)	75%[d]	5 days
Bacillus subtilis	70%[e]	6 days

Different small letters indicate significant difference ($P < 0.05$) among different management strategies.

Discussion

The prevalence and dominance of microorganisms in the Dutse region was examined. Microorganism prevalence and dominance in the Dutse region was studied. The findings of high prevalence of fungal pathogens, *Aspergillus niger* and *Penicillium* species are similar to the literature regarding mango spoilage (Tam & Huyen 2024). They are both known opportunistic pathogens that can colonize fruits; mechanical damage during harvesting and handling is a common way for them to infect (Moreno-Hernandez et al., 2024). They are common in the environment, and under usual post-harvest conditions, it is virtually certain that they will be present in the environment. As per the study by Al-Najada & Al-Suabeyl (2014) *A. niger* causes brown-black rot with white mycelium and black spored surface on the skin of the fruit.

The *Penicillium* species, however, cause blue-mould rot with its watery soft appearance and its bluish-green spores. These fungi are mainly enzymatically degraded. This is due to the presence of extracellular hydrolytic enzymes (cellulase and pectinase) produced by *A. niger* and *Penicillium* species that break down the cell wall components of the fruit tissue, causing the fruit to soften rapidly and develop rot. The infection often initiates at wounds or injured areas on the fruit's surface (Al-Najada & Al-Suabeyl 2014).

These fungi coexist with the presence of *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* indicating a complex microbial ecology on fruit surfaces. The presence of these bacteria and yeasts are probably secondary or synergistic in nature and responsible for a small amount of deterioration in the fruit. Although *Saccharomyces cerevisiae* is most widely recognized as a yeast used for alcohol production, some strains can be spoilage organisms in foods containing high amounts of sugar and low pH levels like fruits and fruit juices (Krisch et al., 2016). Yeasts are very tough, are found in the most extreme environments and are very resistant to heat and conventional preservatives. The main features of spoilage caused by *S. cerevisiae* are off-flavours and visible spoilage characteristics. This yeast has been known to grow quickly, even at refrigerated temperatures, and can continue to compromise the quality of perishable products at any time (Krisch et al., 2016).

Pseudomonas spp., such as *Pseudomonas* spp. *aeruginosa*, are well-known psychrotrophic bacteria that are able to grow and cause spoilage in cold storage (Quintierri et al., 2020). They cause various visible and non-visible symptoms of spoilage such as discoloration, texture loss and off flavors due to the production of heat resistant extracellular proteases and lipases. The bacteria are commonly found in the environment, and can contaminate produce throughout the food supply chain (Raposo et al., 2016).

Virulence of Isolated Pathogens

Results from a pathogenicity test clearly rank the virulence of microorganisms. The highest score (5) obtained for *Aspergillus niger* is not surprising, as the fungus is known to be a key post-harvest pathogen (Al-Najada & Al-Suabeyl 2014). This high virulence can be explained by its production of enzymes that break down the integrity of fruit, including pectinase and cellulase enzymes, which cause rapid and severe decay (Al-Najada & Al-Suabeyl 2014). The same score of 4 is also comparable for *Penicillium* species that are known to cause severe post-harvest rot (Kanashiro et al., 2020).

Also noteworthy is the pathogenicity of *Saccharomyces cerevisiae* (score 3) and *Pseudomonas aeruginosa* (score 3). Less virulent than fungal disease, they may be able to contribute to or cause decay under suitable conditions. *Pseudomonas* species are known to cause cold storage spoilage and their occurrence indicates that they may be a problem in cold stored mangoes where transported or stored in the cold (Quintierri et al., 2020).

Comparative Analysis of Management Strategies

The results of the study on the effectiveness of the management strategies are very important. The advantages of refrigeration at 10°C (90% microbial reduction & shelf life extension to 10 days) validate the fact that refrigeration is one of the most effective interventions to preserve mangoes (Patil et al., 2019). This temperature is in the optimum range for mango storage allowing the deceleration of ripening and respiration process and reducing the growth of most spoilage microorganisms without inducing chilling injury (Nguyen et al., 2023). Temperature control is an essential and very powerful method of maintaining fruit quality. Refrigeration minimized physiological ripening and senescence (e.g., respiration) and generally greatly slowed the proliferation of spoilage microorganisms. The best cold storage temperature for mangoes is 10°C to 12°C, because refrigeration below 10°C may cause chilling injury which may adversely affect the quality of the mangoes (Patil et al., 2019).

The efficacy of sodium benzoate (85% reduction, 7 days extension) is in line with its established fungistatic activity (Kaur et al., 2019). It has the potential to be a viable and cost-effective alternative for small-scale farmers who may not have access to sophisticated refrigeration equipment. Benzoic acid is a widely used preservative which is also fungistatic and commonly used as a food preservative as its sodium salt, sodium benzoate. It is more effective in acidic environments where it turns into benzoic acid, which disrupts the permeability of the cell membranes of microorganisms, preventing the growth of yeasts, moulds and bacteria. It has been proven to inhibit growth of *Aspergillus* species and fungal spoilage in fruits (Kaur et al., 2019).

Likewise, the effectiveness of calcium chloride (75% reduction, 5 day extension) validates its role in strengthening cell wall integrity and thus protecting the fruit against physical and microbial damage (Sajid et al 2019). Modified Atmospheric Packaging (MAP, 80% reduction, 8 day extension) also gave good results and demonstrated the ability of MAP to lower the metabolic rate of the fruit and consequently the rate of spoilage. The results indicate that using low temperatures along with MAP would be an even more powerful method for long distance transportation and for long storage (Ramayya et al. 2012).

Bacillus subtilis is a Gram-positive bacterium, widely recognized as a non-pathogenic organism with beneficial effects for agriculture, both as biostimulant and biocontrol agent. The effectiveness of this agent as a biocontrol is due to its capacity to generate bioactive metabolites, anti-fungal and antibacterial, which form protective biofilms (Rivera-Chaves et al., 2020). It has proven to be an effective inhibitor of a number of fungal pathogens such as *Penicillium* species and *Aspergillus* species (Ban Koffi et al., 2017). The ability of some *B. subtilis* strains to induce systemic resistance in plants further enhances their protective capabilities (Lastochkina et al., 2019).

Implications for Post-Harvest Practices in Nigeria

The result of this research work has application to the post-harvest practices in the Dutse region and other regions. The findings offer a well-defined sequence of mango spoilage control measures. It was established that refrigeration at 10°C is the most effective single intervention and that even small-scale refrigeration could be very effective in reducing loss. The chemical dip using sodium benzoate is also highly effective and readily available to the farmers who don't have access to refrigeration. Calcium chloride can also be used, especially to enhance fruit firmness and overall fruit quality. The biological treatment with *B. subtilis* is a promising and sustainable alternative that can be developed as an eco-friendly biopesticide to comply the global trend of a reduction of synthetic chemicals. The data gives a robust scientific basis for establishing locally-specific post-harvest protocols which could greatly limit mango spoilage, prolong shelf-life and enhance the economic viability of mango production in Nigeria.

Conclusion

The present study was able to isolate and identify the main microbial species responsible for the post-harvest spoilage of ripe mangoes in the study area (Dutse, Jigawa state) as well as assess the efficacy of various practical control measures. *Aspergillus niger* and *Penicillium* species were identified as the most common and harmful fungi which affect post harvesting quality.

The investigation of management strategies showed that refrigeration at 10°C is the most powerful control measure, providing about 90% reduction in microbial growth and the shelf-life was extended by 10 days. The chemical treatments also had tremendous potential and were found to have a high level of protection, and they were an acceptable and low cost option for local farmers. The biological treatment with *Bacillus subtilis* proved to be very effective in its biocontrol potential. Test the effectiveness of the most promising treatments in combination is recommended. Another possible approach would be the use of a biological control agent in conjunction with low temperature storage, with the hope of achieving a synergistic effect, resulting in an even higher reduction in spoilage and shelf life. Such holistic solution might provide a stronger, sustainable solution for the post-harvest value chain.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study design: Mohammad. M; data collection: Nancy. J; analysis and interpretation of results: Mohammad. M, Nancy. J, Idris, A; draft manuscript preparation: Idris A. All authors reviewed the results and approved the final version of the manuscript.

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