IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 5 issue 3 Sept, 2026 Research Article

Post-Harvest Proximate Composition of Infected and Uninfected *Maesobotrya barteri* Fruits from Oduoha-Emohua Forest, Rivers State, Nigeria

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ABSTRACT

Post-harvest microbial infection is a major constraint to the nutritional quality and marketability of indigenous tropical fruits. This study evaluated the proximate composition of *Maesobotrya barteri* (bush berry) fruits infected by *Fusarium equiseti*, *Penicillium vickeryae* and *Bacillus pumilus* relative to uninfected controls harvested from Oduoha-Emohua Forest, Rivers State, Nigeria. Fruits were surface-sterilised, incubated to allow expression of spoilage symptoms, and analysed for moisture, ash, crude protein, crude lipid, crude fibre and carbohydrate using Standard Official Methods of Analysis procedures. Significant differences ($p < 0.05$) were observed across all parameters. Uninfected control fruits retained the highest crude protein (34.98 ± 0.01%) and crude lipid (16.81 ± 0.02%), while infected fruits showed marked reductions protein 23.69–29.23%; lipid 9.23–13.52%. Carbohydrate content increased proportionally in infected samples with *P. vickeryae* up to 42.69 ± 0.04% and crude fibre was highest under *F. equiseti* infection (12.50 ± 0.02%). Moisture content declined sharply in *F. equiseti*-infected fruits (8.60 ± 0.02%). These changes reflect pathogen-driven enzymatic degradation of macromolecules and consequent shifts in the relative contribution of proximate fractions. The results demonstrate that microbial colonisation substantially impairs the nutritional value of *M. barteri* fruits and underscore the need for improved post-harvest handling to preserve this under-utilised indigenous resource.

Keywords: *Maesobotrya barteri*, Post-harvest Infection, Proximate composition, Crude protein, Crude fiber, Nutritional quality.

Introduction

Maesobotrya barteri (Baill.) Hutch. (Phyllanthaceae), commonly called bush berry or bush cherry, is an indigenous rainforest tree widely distributed in southern and eastern Nigeria and other parts of West Africa (Keay, 1989; Ogbuagu and Agu, 2008). Its small, ovoid, blackish-purple fruits are consumed fresh, used in traditional medicine, and hold potential for juice and beverage production (Ogunka-Nnoka *et al*., 2016; Okon *et al*., 2016). Nutritional evaluations have shown that the fruits contain appreciable levels of protein, lipids, fibre and minerals, positioning them as a valuable, if under-exploited, functional food (Ogbuagu and Agu, 2008; Agbagwa *et al*., 2022). Most fleshy tropical fruits such as, *M. barteri* is highly perishable once harvested. High moisture content and progressive tissue softening create favourable conditions for opportunistic bacteria and fungi. These organisms colonise wounds or natural openings during handling and storage (Bridgemohan & Isaac, 2017; González-Estrada *et al.,* 2019). Microbial invasion triggers the secretion of hydrolytic enzymes that degrade host proteins, lipids and structural polysaccharides, leading to measurable changes in proximate composition and progressive loss of nutritional quality (Agrios, 2005; Muhammad *et al*., 2024).

Although earlier studies in Rivers State confirmed the susceptibility of *M. barteri* fruit to microbial spoilage (Agbagwa *et al*., 2022), quantitative data on the magnitude of infection-induced shifts in proximate constituents remain limited. Proximate analysis provides a rapid, standardised assessment of the major nutritional fractions such as moisture, ash, protein, lipid, fibre and carbohydrate, and is therefore a critical tool for evaluating post-harvest quality deterioration.

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Understanding these changes is essential for developing appropriate handling, storage and value-addition strategies that can reduce losses and enhance utilisation of this indigenous forest fruit.

The present study assessed the frequency of occurrence of microbial isolates recovered post-harvest spoilt *M. barteri* and proximate composition (%) of fruits infected and uninfected of *M. barteri* fruits

The findings should supply baseline information on the nutritional impact of microbial infection and contribute to the limited body of knowledge on the post-harvest biology of under-utilised West African forest fruits such as *Maesobotrya barteri* (bush berry).

Materials and Methods

Study Area and Sample Collection

Mature fruits of *M. barteri* were collected from Oduoha-Emohua Forest in Emohua Local Government Area, Rivers State, which lies within the humid tropical rainforest belt of the eastern Niger Delta of Nigeria. Laboratory analyses were performed at the Forest Pathology/Mycology Unit, Department of Forestry and Environment, Rivers State University, Port Harcourt (latitude 4.50°N, longitude 7.00°E; elevation ≈18 m; mean annual temperature ≈27°C; rainfall 2000–2467 mm) (Chukunda, 2014). The plant was taxonomically identified and authenticated by Prof. B. O. Green of the Department of Plant Science and Biotechnology, Rivers State University, Nkpolu Oroworukwo and a voucher specimen was deposited in the herbarium of the Department of Forestry and Environment. The fruits were stored for seven days to observe signs and symptoms of infected parts during storage.

Fruits were held under ambient laboratory conditions for seven days to permit expression of natural spoilage symptoms before processing (Chukunda, 2014).

Isolation and Identification of Microorganisms

The standard blotter method of the International Seed Testing Association (I.S.T.A, 1976) was used for the isolation of the fungal pathogens. *Maesobotrya barteri* seed samples were pre-treated with one percent (1%) Sodium hypochlorite solution for five minutes before peeling the coat of the fruits to remove contaminants on the surface before plating five seeds per Petri-dish. It was incubated in an incubator at 25 ± 2^0C for seven days. One hundred seeds were treated for the post-harvest fruits infection. Pure culture were prepared from the mix culture plates. Identification of the fungi was carried out using stereo-binocular microscope (6-50) based on their habit character and spore characteristics. Where necessary, spores of fungal mycelia and bacterial cells were made on slides and viewed under compound microscope (X_{40}) to confirm the organisms. The identification was done following the description by Barnett and Hunter, (1998; Klich, 2002 and Chukunda *et al.,* 2021).

The percentage disease incidence was calculated using the formula of Rai and Manmatha (2005) and Chukunda (2014).

$$D1 = \frac{D_o}{D} \; x \frac{100}{1} \qquad (1)$$

Where;

D_1 = Disease incidence, D_o = Number of diseased fruits, D = Total number of fruits seed-plated.

Proximate Composition Analysis

The proximate composition of pulp obtained from infected and uninfected fruits was determined in triplicate using standard analytical procedures described by AOAC (2005). Prior to analysis, the fruit pulp samples were thoroughly homogenised to ensure uniformity and representative sampling. Moisture content was determined gravimetrically by drying 2 g of each homogenised sample in a pre-weighed moisture can at 105 °C until a constant weight was attained (AOAC, 2007).

Ash content was determined by incinerating 1 g of the homogenised sample in a muffle furnace at 550 °C for 3 h, or until a light-grey ash of constant weight was obtained. Crude lipid content was determined by Soxhlet extraction of 2 g of the sample using *n*-hexane as the extraction solvent for 3 h.

Following extraction, the solvent was removed, and the extracted lipid was oven-dried at 105 °C to obtain a constant weight (AOAC, 2006).

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Crude protein content was determined using the Kjeldahl method. Approximately 0.1 g of each sample was digested with concentrated sulphuric acid in the presence of an appropriate catalyst, after which the digest was distilled into boric acid solution and titrated against 0.1 N hydrochloric acid. The nitrogen content obtained was converted to crude protein using the conventional nitrogen-to-protein conversion factor of 6.25 (AOAC, 2005).

Crude fibre was determined following sequential digestion of the defatted sample with 1.25% sulphuric acid and 1.25% sodium hydroxide. The resulting residue was dried, weighed, and subsequently ashed, with crude fibre calculated from the loss in weight upon ignition. Total carbohydrate content was estimated by difference according to the equation;

$$Carbohydrate\ (\%) = 100 - (\%Moisture + \%Ash + \%Crude\ Protein + \%Crude\ Lipid + \%Crude\ Fibre)$$

All determinations were performed in triplicate, and the results were expressed on a fresh-weight basis as mean ± standard deviation (AOAC, 2005).

Experimental Design and Statistical Analysis

The experiment was arranged in a Completely Randomised Design (CRD) with three replicates per treatment. Data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics Version 16.0. Where significant differences were detected, means were separated by Duncan's Multiple Range Test (DMRT) at the 5% probability level ($p < 0.05$).

Results

The occurrence of the microbial isolate revealed a total of fifty-three (53) microbial isolates were recovered from spoilt *Maesobotrya barteri* fruits, comprising one bacterial genus (*Bacillus*) and two fungal genera *Fusarium* and *Penicillium*) with the species identified as *Bacillus pumilus Fusarium equiseti*, and *Penicillium vickeryae* respectively. *Bacillus* sp. was the single most frequently encountered organism, accounting for 12 (22.64%) of the 53 isolates (100%), a higher relative frequency are bacterial then any of the three co-occurring fungal isolates (Table 1).

Table 1: Frequency of occurrence of microbial isolates recovered from post-harvest spoilt *Maesobotrya barteri* fruits

Microbial Isolate	Frequency (n)	Relative Frequency (%)
Fusarium equiseti	18	33.96
Penicillium vickeryae	13	24.53
Bacillus pumilus	12	22.64
Control (uninfected)	10	18.87
Total	**53**	**100.00**

The result of the Proximate Composition of Infected and Uninfected *Maesobotrya barteri* berry fruits is as presented in Table 2.

Table 2: Proximate composition (%) of infected and uninfected *Maesobotrya barteri* fruits

Sample	Proximate composition of infected and uninfected *Maesobotrya barteri* ruits					
	Ash (%)	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Crude Fibre (%)	Carbohydrate (%)
Fusarium equiseti	2.74 ± 0.02^{c}	8.60 ± 0.02^{c}	29.23 ± 0.58^{b}	13.52 ± 0.48^{b}	12.50 ± 0.02^{a}	33.70 ± 0.44^{c}
Penicillium vickeryae	2.40 ± 0.02^{d}	9.70 ± 0.02^{b}	25.68 ± 0.01^{c}	10.72 ± 0.01^{c}	8.81 ± 0.02^{c}	42.69 ± 0.04^{a}
Bacillus pumilus	3.62 ± 0.01^{a}	15.85 ± 0.07^{a}	23.69 ± 0.02^{d}	9.23 ± 0.02^{d}	10.65 ± 0.01^{b}	36.87 ± 0.08^{b}
Control (uninfected)	3.47 ± 0.01^{b}	15.85 ± 0.01^{a}	34.98 ± 0.01^{a}	16.81 ± 0.02^{a}	7.91 ± 0.03^{d}	21.04 ± 0.06^{d}

Note: Means ±STD within the same column followed by the same superscript letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

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The proximate constituents differed significantly ($p < 0.05$) among the four fruit pathogens isolated from the fruits. Uninfected control fruits recorded the highest crude protein (34.98 ± 0.01%) and crude lipid (16.81 ± 0.02%). Infection by other three microorganisms reduced both fractions: protein declined to 29.23 ± 0.58% under *F. equiseti*, 25.68 ± 0.01% under *P. vickeryae* and 23.69 ± 0.02% under *B. pumilus*; lipid declined to 13.52 ± 0.48%, 10.72 ± 0.01% and 9.23 ± 0.02%, respectively. Carbohydrate content exhibited the opposite trend, reaching its maximum in *P. vickeryae*-infected fruits (42.69 ± 0.04%) and the lowest was the control (21.04 ± 0.06%). Crude fibre was highest in *F. equiseti*-infected fruits (12.50 ± 0.02%) and lowest in the control (7.91 ± 0.03%). Moisture content was identical in the control and *B. pumilus*-infected fruits (15.85%) but fell sharply under *F. equiseti* infection (8.60 ± 0.02%). Ash content ranged from 2.40 ± 0.02% (*P. vickeryae*) to 3.62 ± 0.01% (*B. pumilus*).

Discussion

This study has revealed some of the microorganisms associated with the spoilage of *Maesobotrya barteri* (bush berry) harvested from Oduoha-Emohua Forest, Rivers State, Nigeria and their impact on the proximate composition and hence nutritional quality of the fruit. The recovery of *Bacillus* sp. as the single most frequent isolate (22.64%) among the post-harvest microflora of *M. barteri* fruit is consistent with the well-documented ubiquity and environmental resilience of this genus. As spore-forming organisms capable of surviving desiccation and fluctuating temperature, *Bacillus* species are commonly recovered from the surfaces of soft, perishable fruits during harvesting, handling and marketing, and have been reported elsewhere as a recurrent component of the post-harvest microbiota of such produce, functioning either as opportunistic spoilage agents or as antagonists of co-occurring fungal pathogens depending on the local microbial community structure (He et al., 2020). It is instructive that this finding diverges markedly from the only previously published account of post-harvest *M. barteri* microbiology, in which Agbagwa *et al.* (2022) isolated exclusively yeast organisms (*Candida* sp. and *Saccharomyces*) from spoilt bush cherry fruit obtained elsewhere within Rivers State, with no *Bacillus* or filamentous mould recorded. This disparity, despite both studies being conducted on the same host fruit within the same general locality, is most plausibly explained by differences in the degree or stage of fruit spoilage sampled, seasonal variation in ambient microbial load, differences in the specific micro-environments and markets from which fruits were sourced, and differences in isolation media and incubation conditions, since yeasts and moulds/bacteria often display differential selectivity for nutrient-rich, high-sugar substrates such as the succulent pulp of an over-ripened fruit (Kaya, et al., 2008) This underscores that the post-harvest microflora of a given fruit species is not fixed, but is instead shaped by the interplay of intrinsic fruit factors and extrinsic environmental and handling conditions.

Results of the proximate composition of infected and uninfected of *Maesobotrya barteri* fruits revealed a pronounced decline in crude protein and crude lipid in infected fruits is attributable to the extracellular proteolytic and lipolytic enzymes secreted by the colonising microorganisms. These enzymes hydrolyse host proteins and triglycerides, mobilising amino acids and fatty acids for microbial growth and simultaneously depleting the nutritional reserves of the fruit tissue (Agrios, 2005; Dean et al., 2012). *Fusarium* species are especially noted for the production of a broad suite of cell-wall-degrading and proteolytic enzymes that facilitate rapid tissue maceration (Dean et al., 2012), which explains the intermediate protein retention observed under *F. equiseti* relative to the more severe reductions caused by *P. vickeryae* and *B. pumilus*. This is consistent with the present finding (Chukunda and Offor, 2015).

The reciprocal increase in carbohydrate content is best interpreted as a proportional consequence of protein and lipid loss rather than an absolute rise in carbohydrate biosynthesis. When protein and lipid fractions diminish, the relative contribution of the residual carbohydrate fraction to total dry matter automatically increases (Bhat et al., 2012). In addition, partial enzymatic depolymerisation of structural polysaccharides may release analytically detectable soluble carbohydrates, further elevating the measured carbohydrate value (Obinna-Echem and Chukunda, 2018).

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Elevation of crude fibre under *F. equiseti* infection may reflect two complementary processes preferential degradation of non-fibrous cell-wall components that leaves a higher proportion of acid- and alkali-resistant material, and deposition of fungal cell-wall constituents basically chitin and β-glucans) that remain recoverable under standard crude-fibre protocols (Shahidi and Naczk, 2004). The sharp reduction in moisture content associated with *F. equiseti* is consistent with extensive mycelial colonisation that disrupts cellular integrity and accelerates water loss from the fruit tissue as earlier reported by Chukunda et al (2023). Uninfected fruits maintain superior levels of crude protein and crude lipid, whereas infected fruits exhibit progressive depletion of these fractions, compensatory increases in carbohydrate, and pathogen-specific changes in fibre and moisture. The observed changes are consistent with enzymatic degradation of host macromolecules and constitute a measurable loss of nutritional quality (Álvarez-Martínez *et al.,* 2021)

Collectively, these proximate shifts demonstrate that post-harvest infection does not merely cause visible decay; but systematically redistributes the major nutritional fractions of *M. barteri* fruit. The magnitude of protein and lipid loss is particularly consequential because these macronutrients contribute substantially to the fruit's value as a functional food. The results therefore reinforce the necessity of minimising mechanical injury and microbial contamination during harvesting, transport and storage if the nutritional potential of this indigenous species is to be retained.

Conclusion

Microbial infection by *Fusarium equiseti*, *Penicillium vickeryae* and *Bacillus pumilus* significantly alters the proximate composition of *Maesobotrya barteri* fruits and hence the nutritional quality. Uninfected fruits maintain superior levels of crude protein and crude lipid, whereas infected fruits exhibit progressive depletion of these fractions, compensatory increases in carbohydrate, pathogen-specific changes in fibre and moisture. These changes are consistent with enzymatic degradation of host macromolecules and constitute a measurable loss of nutritional quality. Effective post-harvest handling remains essential to preserve the food and economic value of this under-utilised forest fruit.

Recommendations

1. Fruit handlers and marketers should adopt careful harvesting techniques that minimise mechanical injury, together with prompt sorting and sanitation, to reduce opportunities for microbial colonisation.
2. Storage under cool, well-ventilated conditions should be encouraged to slow the progression of infection and limit further proximate deterioration.
3. Fruits showing advanced decay should be excluded from fresh consumption or processing streams to safeguard overall product quality.
4. Future studies should examine the kinetics of proximate change under controlled storage temperatures and evaluate simple botanical or physical treatments capable of retarding infection-related nutrient loss.

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