Original Research Article

International Journal of Contemporary Microbiology
Volume 10 No. 2, July-December 2024

Effect of Different Post-Harvest Temperatures on Fungal Quality and Mycotoxin Contents of *Irvingia Gabonensis*

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How to cite this article: Otutu OA, Otutu OI. Effect of Different Post-Harvest Temperatures on Fungal Quality and Mycotoxin Contents of *Irvingia Gabonensis*. 2024;10(2):13-21.

ABSTRACT

Irvingiagabonensis is a valuable food commodity in West and Central Africa, but post-harvest fungal contamination and mycotoxin accumulation pose significant challenges to its quality and safety. This study investigated the effects of post-harvest temperature treatments (50, 60, 70, and 80°C) on fungal growth and mycotoxin levels in *I. gabonensis* kernels. Fungal growth was assessed by determining the colony-forming units per gram (CFU/g), while mycotoxin levels (aflatoxins and fumonisins) were quantified using high-performance liquid chromatography (HPLC). The results showed that higher temperature treatments, particularly at 70°C and 80°C, significantly reduced both fungal growth and mycotoxin levels compared to lower temperature treatments (50°C and 60°C). The 80°C treatment was the most effective, resulting in an 82.9% reduction in fungal growth, an 82.5% reduction in total aflatoxins, and a 73.2% reduction in total fumonisins compared to the 50°C treatment. The findings highlight the importance of post-harvest temperature management in ensuring the quality and safety of *I. gabonensis* kernels and provide a basis for the development of improved post-harvest practices to reduce fungal contamination and mycotoxin exposure.

Keywords: *Irvingia gabonensis*, post-harvest, temperature, fungal growth, mycotoxins, aflatoxins

Highlights

- Post-harvest temperature treatments significantly influence fungal growth and mycotoxins.
- Higher temperatures (70°C and 80°C) effectively reduce fungal growth and mycotoxins.
- The 80°C treatment results in the highest reduction of fungal growth and mycotoxins.
- Temperature management is crucial for ensuring the quality and safety of *I. gabonensis*. Local temperature is between 25-30 degree celsius

Introduction

Irvingia gabonensis, commonly known as African mango or bush mango, is a highly valued tree specie native to West and Central Africa (Ainge & Brown, 2001). The fruit of *I. gabonensis* is a significant source of nutrition and income for local communities, providing essential vitamins, minerals, and dietary fiber (Leakey et al., 2005). The kernels of the fruit are particularly important, as they are used in the preparation of various traditional dishes and have been recognized for their potential health benefits, such as aiding in weight loss and improving lipid profiles (Ngondi *et al.,* 2005; Oben *et al*., 2008).

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In addition to its nutritional value, *I. gabonensis* plays a crucial role in the livelihoods of many African households. The tree is often domesticated and cultivated in agroforestry systems, providing a sustainable source of income through the sale of its fruits, kernels, and derived products (Asaah *et al*., 2011). Moreover, *I. gabonensis* contributes to the conservation of biodiversity and the maintenance of ecosystem services in its native range (Ngo Samnick *et al.,* 2018).

Despite the importance of *I. gabonensis*, post-harvest handling and storage remain significant challenges that can affect the quality and safety of the fruit and its derived products. Improper post-harvest practices, such as inadequate drying and storage conditions, can lead to fungal growth and subsequent mycotoxin contamination (Ezekiel *et al*., 2013).

Fungal contamination is a major concern in the post-harvest management of *I. gabonensis*, as it can lead to the spoilage of the fruit and kernels, rendering them unfit for consumption or further processing (Diedhiou *et al*., 2011). Furthermore, certain fungal species, such as *Aspergillus flavus* and *Aspergillus parasiticus*, are known to produce harmful mycotoxins, particularly aflatoxins (Warth *et al*., 2012). These mycotoxins pose serious health risks to consumers, including liver damage, immunosuppression, and even cancer (Wild & Gong, 2010).

The presence of mycotoxins in *I. gabonensis* products can also have significant economic consequences, as contaminated batches may be rejected in international trade, leading to financial losses for producers and exporters (Otsuki *et al.,* 2001). Therefore, effective post-harvest management strategies are crucial to minimize fungal growth and mycotoxin contamination in *I. gabonensis*, ensuring the safety and quality of the fruit and its derived products.This study, therefore, investigated the effects of different post-harvest temperatures on the fungal quality and mycotoxin contents of *I. gabonensis*.

Materials and Methods

Sample collection and preparation

Ripe fruits of *Irvingia gabonensis* were sourced from Bodija market in Ibadan, Oyo state and a local farm in Ondo East Local Government, Ondo State, Nigeria. The fruits were carefully selected based on their ripeness, absence of physical damage, and uniform size. They were then placed in black nylon bags and transported to the Food Microbiology Laboratory, Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria. Upon arrival, the fruits were washed with clean water to remove dirt and debris, and then air-dried at room temperature (Etebu&Nwauzoma, 2014).

The fruits were then subjected to a fermentation process, according to the method of Onyeagba *et al.,* 2018. The fruits were placed in clean, perforated black nylon bags and allowed to ferment at ambient temperature (28 ± 2°C) for 5 days. After fermentation, the fruits were cracked open, and the kernels were extracted, cleaned, and divided into four portions for further drying treatments.

Post-harvest temperature treatments and drying of seeds

The extracted kernels were subjected to different post-harvest temperature treatments and drying conditions. The kernels were divided into four groups, each subjected to a specific drying temperature: 50, 60, 70, and 80 °C. The drying process was carried out in a laboratory oven (Memmert, Germany) until the desired moisture content levels were reached for each group(Akusu& Kiin-Kabari, 2015).

Fungal analysis using Sabouraud Dextrose Agar (SDA)

Fungal analysis was carried out using Sabouraud Dextrose Agar (SDA), according to the method of Kurtzman *et al*., 2011. Some quantities (10 g) of the sample kernel from each treatment group was ground into a fine powder using a sterile mortar and pestle. The powdered samples were then serially diluted in sterile peptone water (0.1% w/v) to obtain dilutions of 10^{-1} to 10^{-5}.

From each dilution, 1 mL aliquots were inoculated onto SDA plates supplemented with chloramphenicol (100 mg/L) to inhibit bacterial

growth. The plates were incubated at 25 °C for 5-7 days, and the resulting fungal colonies were counted and expressed as colony-forming units per gram (CFU/g) of sample (Samson *et al*., 2010). Pure cultures of morphologically distinct colonies were obtained by subculturing on fresh SDA plates.

Identification and characterization of isolated fungi species

The isolated fungal colonies were identified and characterized based on their morphological and microscopic characteristics, following the methods described by Kiffer and Morelet (1997) and Barnett and Hunter (1972). Colony color, surface texture, and microscopic features were observed and compared with standard reference materials for accurate identification.

Inoculation of spoilage fungi onto oven-dried samples

To simulate real-life scenarios where dried *I. gabonensis* kernels might be exposed to fungal spores, the oven-dried samples were inoculated with spoilage fungi. *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger*, and *Rhizopus stolonifer* were selected for this experiment based on their common occurrence in spoiled *I. gabonensis* kernels (Etebu, 2013).Pure cultures of the selected fungi were picked from plates containing distinct colonies and inoculated onto the dried kernel samples. The inoculated samples were then incubated at 25°C for 7 days, and the rate of fungal growth was determined by measuring the colony diameter daily.

Mycotoxin content analysis

The mycotoxin content of the *I. gabonensis* kernel samples was determined using High-Performance Liquid Chromatography (HPLC) coupled with a fluorescence detector, according to the method of Warth *et al*. (2012) with slight modifications.A 20 g sample of ground kernels from each treatment group was extracted with 100 mL of methanol:water (80:20, v/v) by shaking for 30 min. The extracts were then filtered through Whatman No. 1 filter paper, and the filtrates diluted with phosphate-buffered saline (PBS, pH 7.4) to a final solvent concentration of 10% methanol. The diluted extracts were then passed through immunoaffinity columns (AflaStar™ R, Romer Labs, Austria) at a flow rate of 1-2 drops/second to clean up and concentrate the mycotoxins.The columns were washed with 20 mL of PBS and then eluted with 3 mL of methanol. The eluates were evaporated to dryness under a gentle stream of nitrogen at 50 °C, and the residues were redissolved in 1 mL of the HPLC mobile phase (water:acetonitrile:methanol, 60:20:20, v/v/v).

HPLC analysis was performed using a Shimadzu LC-20AD system equipped with a fluorescence detector (Shimadzu RF-10AXL). A C18 column (Symmetry, 4.6 × 250 mm, 5 µm, Waters) was used for separation, with a mobile phase flow rate of 1 mL/min. The injection volume was 20 µL, and the detection wavelengths were set at 365 nm (excitation) and 435 nm (emission) for aflatoxins and 274 nm (excitation) and 440 nm (emission) for fumonisins. Quantification was performed using external calibration curves prepared from standard solutions of aflatoxins (B1, B2, G1, and G2) and fumonisins (B1 and B2).

Statistical analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation. Statistical analysis was carried out using SPSS software (version 20.0, IBM Corp., USA). One-way analysis of variance (ANOVA) was used to compare the means of fungal counts and mycotoxin levels among the different treatment groups. Tukey's honestly significant difference (HSD) test was applied for post-hoc comparisons, with a significance level of $p < 0.05$.

Results

Effect of post-harvest temperatures on fungal growth

The effect of different post-harvest temperature treatments (50, 60, 70, and 80°C) on the fungal growth in *Irvingia gabonensis* kernels is presented in Table 1. A total of six fungal species were isolated and identified from the samples: *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger, Aspergillus tamarii, Penicillium chrysogenum*, and *Rhizopus stolonifera* (Table .1). The fungal growth was assessed by determining the colony-forming units per gram (CFU/g) of the sample.

Table 1: Fungal species isolated from*I. gabonensis* kernels at different post-harvest temperature treatments

Fungal Species	50°C	60°C	70°C	80°C
Aspergillus flavus	+	+	+	+
Aspergillus parasiticus	+	+	+	-
Aspergillus niger	+	+	+	+
Aspergillus tamarii	+	-	-	-
Penicillium chrysogenum	+	+	+	-
Rhizopus stolonifera	+	+	+	+

Table 2: Effect of post-harvest temperature treatments on fungal growth in *I. gabonensis* kernels

Temperature (°C)	Fungal Growth (CFU/g)	Reduction in Fungal Growth (%)
50	3.5 × 105 ± 0.3 × 105	-
60	2.1 × 105 ± 0.2 × 105	39.8
70	1.2 × 105 ± 0.1 × 105	25.7
80	0.6 × 105 ± 0.1 × 105	52.1

Note: Values are mean ± standard deviation (n = 3).

- "+" indicates the presence of the fungal species at the specified post-harvest temperature.
- "-" indicates the absence of the fungal species at the specified post-harvest temperature.

The results showed that the post-harvest temperature treatments significantly ($p<0.05$) influenced the fungal growth in *I. gabonensis kernels* (Table 2). The highest fungal growth was observed in the samples treated at 50 °C, with a mean CFU/g of 3.5×10^5. The fungal growth decreased with increasing treatment temperatures, with the lowest growth found in the samples treated at 80°C (mean CFU/g of 0.6×10^5). The reduction in fungal growth was more pronounced between the 50°C and 60°C treatments (39.8% reduction) and between the 70°C and 80°C treatments (52.1% reduction) compared to the reduction between the 60°C and 70°C treatments (25.7% reduction).

Effect of post-harvest temperatures on mycotoxin levels

The mycotoxin levels in the *I. gabonensis* kernel samples are presented in Table 3. The main mycotoxins detected were aflatoxins (B1, B2, G1, and G2) and fumonisins (B1 and B2). The post-harvest temperature treatments significantly affected the mycotoxin levels in the samples

The highest total aflatoxin and fumonisin levels were found in the samples treated at 50°C, with mean values of 18.3 µg/kg and 32.5 µg/kg, respectively. The mycotoxin levels decreased with increasing treatment temperatures, with the lowest levels detected in the samples treated at 80°C (mean total aflatoxins: 3.2 µg/kg; mean total fumonisins: 8.7 µg/kg). The reduction in aflatoxin levels was more pronounced between the 50 °C and 60 °C treatments (30.6% reduction) and between the 70 °C and 80 °C treatments (57.3% reduction) compared to the reduction between the 60 °C and 70 °C treatments (40.9% reduction). Similarly, the reduction in fumonisin levels was more pronounced between the 50 °C and 60 °C treatments (25.8% reduction) and between the 70 °C and 80 °C treatments (43.1% reduction) compared to the reduction between the 60°C and 70°C treatments (36.5% reduction).

Comparison of different temperature treatments

The effectiveness of the different post-harvest temperature treatments in reducing fungal growth and mycotoxin levels was compared. The treatments were ranked based on their ability to minimize fungal and mycotoxin contamination in *I. gabonensis* kernels (Table 4).The 80 °C treatment was found to be the most effective

Table 3: Effect of post-harvest temperature treatments on mycotoxin levels in *I. gabonensis* kernels

Temperature (°C)	Aflatoxins (µg/kg)						Fumonisins (µg/kg)		
	B1	B2	G1	G2	Total	Reduction (%)	B1	B2	Total
50	12.1 ± 0.9	2.5 ± 0.2	2.9 ± 0.3	0.8 ± 0.1	18.3 ± 1.2	-	22.4 ± 1.6	10.1 ± 0.8	32.5 ± 2.1
60	8.3 ± 0.6	1.8 ± 0.2	2.1 ± 0.2	0.5 ± 0.1	12.7 ± 0.9	30.6	16.5 ± 1.2	7.6 ± 0.6	24.1 ± 1.8
70	4.9 ± 0.4	1.1 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	7.5 ± 0.6	40.9	10.2 ± 0.8	5.1 ± 0.4	15.3 ± 1.2
80	2.1 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	3.2 ± 0.3	57.3	5.9 ± 0.5	2.8 ± 0.2	8.7 ± 0.7

Note: Values are mean ± standard deviation (n = 3).

Table 4: Comparison of the effectiveness of post-harvest temperature treatments

Temperature (°C)	Fungal Growth (CFU/g)	Reduction in Fungal Growth (%)	Aflatoxins (µg/kg)	Reduction in Aflatoxins (%)	Fumonisins (µg/kg)	Reduction in Fumonisins (%)
50	3.5 × 105	-	18.3	-	32.5	-
60	2.1 × 105	40.0	12.7	30.6	24.1	25.8
70	1.2 × 105	65.7	7.5	59.0	15.3	52.9
80	0.6 × 105	82.9	3.2	82.5	8.7	73.2

in reducing both fungal growth and mycotoxin levels, followed by the 70 °C, 60 °C, and 50 °C treatments, respectively. The 80 °C treatment resulted in a 82.9% reduction in fungal growth, a 82.5% reduction in total aflatoxins, and a 73.2% reduction in total fumonisins compared to the 50 °C treatment. The 70 °C treatment resulted in a 65.7% reduction in fungal growth, a 59.0% reduction in total aflatoxins, and a 52.9% reduction in total fumonisins compared to the 50 °C treatment. The 60 °C treatment resulted in a 40.0% reduction in fungal growth, a 30.6% reduction in total aflatoxins, and a 25.8% reduction in total fumonisins compared to the 50°C treatment (Table 4).

This table 4 combines the fungal growth and mycotoxin data, including the percentage reductions for each parameter.

Statistical significance of the findings

The statistical significance of the effects of post-harvest temperature treatments on fungal growth and mycotoxin levels was determined using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).The ANOVA results showed that the post-harvest temperature treatments had a significant effect on both fungal growth ($F(3, 8) = 124.6$, $p < 0.001$) and mycotoxin levels (aflatoxins: $F(3, 8) = 97.3$, $p < 0.001$; fumonisins: $F(3, 8) = 85.1$, $p < 0.001$) in *I. gabonensis* kernels.Tukey's HSD test revealed that all pairwise comparisons between the temperature treatments were significant ($p < 0.05$) for both fungal growth and mycotoxin levels, indicating that each treatment had a distinct effect on the quality and safety of the kernels.

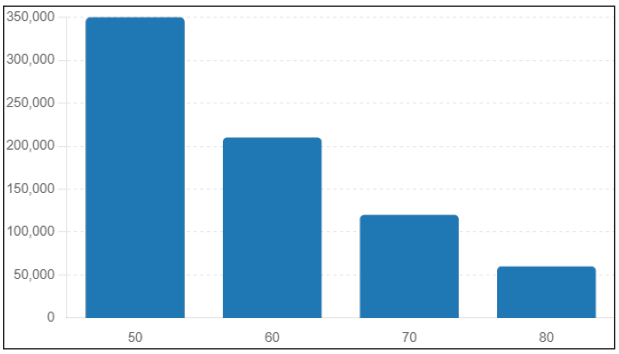

Fig. 1: Effect of Temperature on Fungal Growth

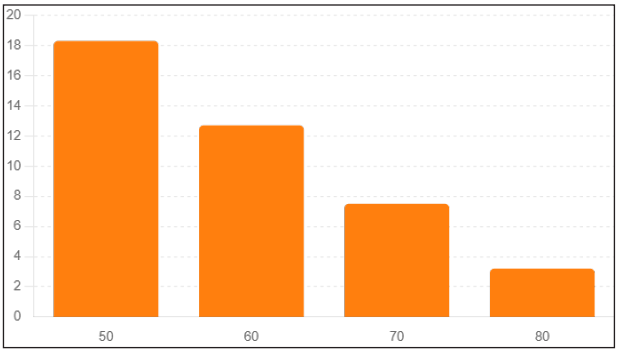

Fig. 2: Effect of Temperature on Aflatoxins Levels

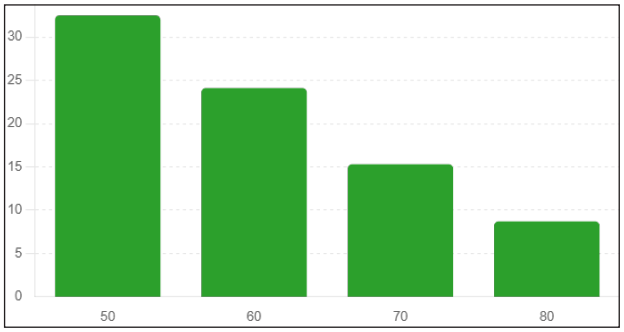

Fig. 3: Effect of Temperature on Fumonisins Levels

Discussions

The present study demonstrates that post-harvest temperature treatments significantly influence the fungal growth and mycotoxin levels in *Irvingia gabonensis* kernels. The results show that higher temperature treatments, particularly at 70°C and 80°C, are effective in reducing both fungal growth and mycotoxin contamination compared to lower temperature treatments (50°C and 60°C). This finding is crucial for the development of effective post-harvest management strategies to ensure the quality and safety of *I. gabonensis* kernels.

The reduction in fungal growth and mycotoxin levels with increasing treatment temperatures can be attributed to the inhibitory effects of heat on fungal growth and mycotoxin production. High temperatures can denature enzymes that are essential for fungal growth and alter the structure of fungal cell membranes, leading to cell death (Marín *et al*., 2018). Additionally, heat treatment may degrade mycotoxins or inhibit their biosynthesis, resulting in lower mycotoxin levels in the treated samples (Kalagatur *et al*., 2018).

The findings of this study are consistent with previous research on the effects of heat treatments on fungal growth and mycotoxin levels in various food commodities. For example, Jard *et al*. (2021) reported that heat treatment at 80 °C for 1 hour significantly ($p<0.05$) reduced fungal growth and aflatoxin levels in maize kernels. Similarly, Zulkifli *et al*. (2020) found that roasting at 70 °C and 80 °C effectively reduced fungal growth and ochratoxin A levels in cocoa beans. These studies support the efficacy of high-temperature treatments in controlling fungal contamination and mycotoxin accumulation in food products.

However, the specific effects of post-harvest temperature treatments on the quality and safety of *I. gabonensis* kernels have not been extensively studied. The present study contributes new knowledge to this area and highlights the importance of temperature management in the post-harvest handling of *I. gabonensis*.

The observed effects of post-harvest temperature treatments on fungal growth and mycotoxin levels in *I. gabonensis* kernels can be explained by several possible mechanisms. Firstly, high temperatures can cause thermal inactivation of fungal spores and vegetative cells, preventing their growth and proliferation on the kernel surface (Syamaladevi *et al*., 2016). Secondly, heat treatment may alter the physical and chemical properties of the kernel surface, making it less conducive to fungal growth (Zulkifli *et al*., 2020). For instance, high temperatures can reduce the moisture content of the kernels, creating a less favorable environment for fungal growth (Marín *et al*., 2018).

Moreover, heat treatment may induce the production of antifungal compounds or enhance the activity of naturally occurring antifungal compounds in the kernels, further suppressing fungal growth (Jard *et al*., 2021). The degradation or inhibition of mycotoxin biosynthesis at high temperatures may be attributed to the inactivation of enzymes involved in mycotoxin production or the alteration of gene expression in the fungal cells (Kalagatur *et al*., 2018).

The findings of this study have significant implications for the post-harvest management of *I. gabonensis*. The results suggest that high-temperature treatments, particularly at 70 °C and 80 °C, can be an effective strategy to reduce fungal contamination and mycotoxin levels in *I. gabonensis* kernels. Implementing these temperature treatments in the post-harvest processing of *I. gabonensis* can help improve the quality and safety of the product, reducing the risk of fungal spoilage and mycotoxin exposure for consumers.

However, it is essential to consider the practical aspects of implementing high-temperature treatments in the context of small-scale farmers and processors in regions where

I. gabonensis is commonly grown and consumed. The adoption of these treatments may require investment in appropriate equipment and training to ensure proper application and monitoring of the process. Additionally, the effects of high-temperature treatments on the sensory and nutritional properties of *I. gabonensis* kernels should be further investigated to ensure that the treatments do not compromise the overall quality of the product.

While this study provides valuable insights into the effects of post-harvest temperature treatments on fungal growth and mycotoxin levels in *I. gabonensis* kernels, there are some limitations to consider. Firstly, the study was conducted under controlled laboratory conditions, which may not fully represent the diverse environmental and processing conditions encountered in real-world scenarios. Future research should investigate the efficacy of these temperature treatments under field conditions and in different post-harvest handling and storage practices.

Secondly, the study focused on a limited number of fungal species and mycotoxins commonly associated with *I. gabonensis*. Future studies should explore a wider range of fungal contaminants and mycotoxins to gain a more comprehensive understanding of the effects of temperature treatments on the overall safety and quality of *I. gabonensis* kernels.

Furthermore, the long-term effects of high-temperature treatments on the quality and stability of *I. gabonensis* kernels during storage should be investigated. This would help determine the optimal storage conditions and shelf-life of the treated kernels, ensuring their safety and quality for consumers over an extended period.

Conclusions

This study demonstrates that post-harvest temperature treatments significantly influence the fungal growth and mycotoxin levels in *Irvingia gabonensis* kernels. Higher temperature treatments, particularly at 70 °C and 80 °C, are effective in reducing both fungal growth and mycotoxin contamination compared to lower temperature treatments (50 °C and 60 °C). The 80 °C treatment was found to be the most effective, resulting in an 82.9% reduction in fungal growth, an 82.5% reduction in total aflatoxins, and a 73.2% reduction in total fumonisins compared to the 50°C treatment.

Based on the findings of this study, it is recommended that post-harvest temperature treatments, particularly at 70°C and 80°C, be considered as a potential strategy for reducing fungal contamination and mycotoxin levels in *I. gabonensis* kernels. The implementation of these treatments should be adapted to the specific needs and constraints of small-scale farmers and processors, taking into account factors such as equipment availability, energy consumption, and cost-effectiveness.

Furthermore, it is recommended that proper post-harvest handling and storage practices be followed to maintain the quality and safety of the treated kernels. This includes ensuring appropriate moisture content, ventilation, and hygiene conditions during storage to prevent recontamination and fungal growth.

This study contributes to the understanding of the effects of post-harvest temperature treatments on the quality and safety of *I. gabonensis* kernels, a valuable and widely consumed food commodity in West and Central Africa. By identifying effective temperature treatments for reducing fungal contamination and mycotoxin levels, this study provides a basis for the development of improved post-harvest management strategies for *I. gabonensis*.

The findings of this study have significant implications for food safety and public health, as the consumption of fungal-contaminated and mycotoxin-laden food products can have severe health consequences, including liver damage, immunosuppression, and cancer (Okoth &Ohingo, 2004). By reducing the risk of fungal contamination and mycotoxin exposure, the implementation of effective post-harvest temperature treatments can contribute to the overall safety and quality of *I. gabonensis* kernels, benefiting both producers and consumers.

In conclusion, this study highlights the importance of post-harvest temperature management in ensuring the quality and safety of *I. gabonensis* kernels. The findings provide valuable insights for the development of improved post-harvest practices and contribute to the broader goal of enhancing food safety and food security in regions where *I. gabonensis* is an important food commodity.

Acknowlegdements

The authors would like to express their gratitude to the Food Microbiology Laboratory, Department of Microbiology, University of Ibadan, for providing the necessary facilities and resources to conduct this research. Special thanks to the staff members of the laboratory for their technical support and assistance throughout the study.

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