Comparative Effect of Bio-Preservatives and Chemical Preservative on Microbial Load of Stored Dika Kernel Flour

RESEARCH ARTICLE

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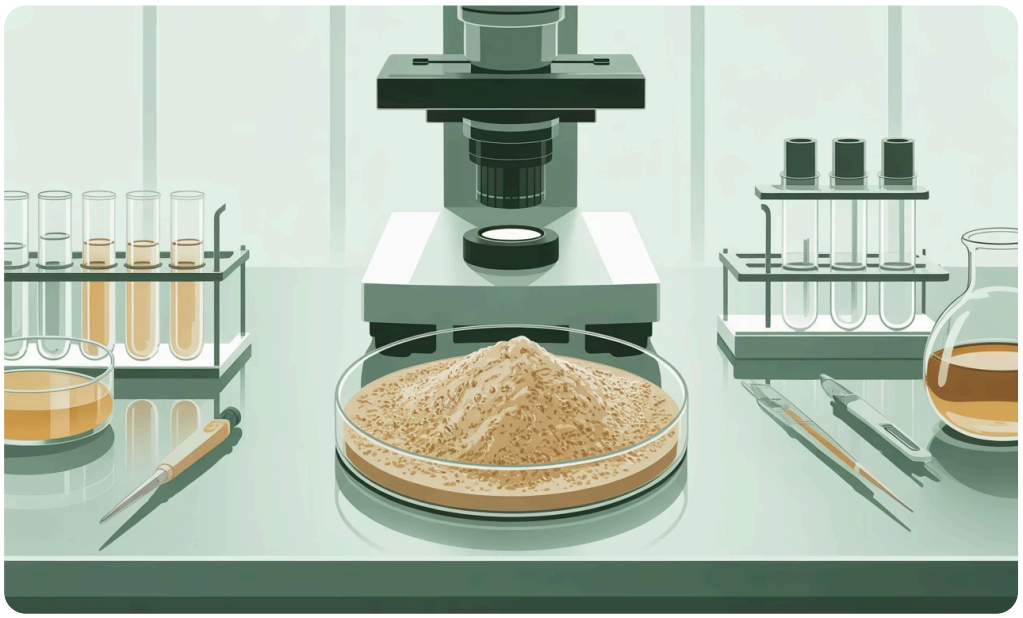
This article is part of a special issue titled Sustainability, innovation, and development: A Festschrift in honour of Rt. Rev. Prof. Obeka Samuel Sunday.



ABSTRACT

The study evaluated the bacterial and fungal load of dika kernel flour preserved with ginger and ginger extract. This was with the aim of extending the shelf life of dika kernel flour. Ginger powder and crude polyphenol extract were obtained from dried ginger. Ginger extract, ginger powder, and butylated hydroxytoluene were incorporated as preservatives in dika kernel flour at 7.5%, 7.5%, and 2.5% (w/w), respectively. Total viable and total fungal counts of dika kernel flour were determined using the pour-plate method.

The total viable count of dika kernel flour preserved with biopreservatives ranged from 0.56 – 1.65 log cfu/g (week 0) to 4.01 – 4.56 log cfu/g (week 12). The total viable count for chemical preservative (BHT) increased from 1.49 to 4.31 log cfu/g. The fungal count of dika kernel flour for biopreservatives ranged from 1.23 – 1.65 log cfu/g (week 0) to 3.94 – 4.45 log cfu/g (week 12). The fungal count of dika kernel flour preserved with BHT ranged from 1.41 – 4.26 log cfu/g (week 12). Statistical analysis revealed significant reductions in total bacterial (Cohen's $d = 0.78$, $p < 0.01$) and fungal counts (Cohen's $d = 0.71$, $p < 0.01$) in samples with preservatives compared with unpreserved controls, indicating a strong positive effect of the preservatives.

The study concluded that ginger powder and ginger extract effectively preserved dika kernel flour, achieving a mean reduction in microbial load of 0.96 log cfu/g (95% CI: 0.85-1.07) for bacteria and 0.51 log cfu/g (95% CI: 0.42-0.60) for fungi compared to controls throughout the 12-week storage period.

Methodology

Pour-plate technique for microbial analysis of preserved dika kernel flour over 12 weeks

Key Variables

Ginger powder, ginger extract, and BHT as preservatives at different concentrations

Main Finding

Significant reduction in bacterial and fungal counts with biopreservatives compared to controls

Keywords: Bio-preservatives, dika kernel flour, ginger extract, microbial load, food preservation, shelf life

INTRODUCTION

Microorganisms cause spoilage, reducing the quality and quantity of processed food (Anwar et al., 2009). Spoilage can occur at any stage, from raw material purchase to consumption, including processing, packaging, distribution, retail display, transport, storage, and consumer use. Effective control at these stages aims to ensure satisfactory shelf life, high quality, and safety. Spoilage can be caused by physical, chemical, enzymatic, and microbiological reactions (Doyle, 2009).

Microbial spoilage alters the nutritional composition of seeds and grains, reducing their food value (Appert, 1987; Ofuya and Lale, 2001). Storage fungi are a known cause of seed and grain deterioration, with biopreservation using natural antimicrobials emerging as a promising alternative to chemical methods (Agarwal and Sinclair, 1997; Mutters, 1998; Murray, 2000). These fungi lower seed germination, discolour seeds, and produce mycotoxins, which can be found in high concentrations in seeds. Common storage fungi include various species of Aspergillus and Penicillium (Mutters, 1998).

01

Initial Contamination

Food spoilage begins during processing, with microbial contamination from soil, handlers, and equipment.

02

Favourable Storage

Extended storage promotes fungal growth, especially for Aspergillus and Penicillium species.

03

Quality Loss

Microbial spoilage reduces nutritional value, causes mycotoxin production, and raises safety concerns.

Ikhatua et al. (2010) identified seven fungal isolates associated with dika kernels: Aspergillus fumigatus, Aspergillus niger, Asperoporium spp., Pestalotia spp., Penicillium spp., and Mucor spp. Aspergillus fumigatus was the most prevalent and was identified as a spoilage pathogen of Irvingia kernels due to its ability to grow above 40 °C and in low moisture content (Huis and Veld, 1996), making it a significant storage fungus.

Microbiological spoilage is largely preventable using various preservation techniques that inhibit microbial growth (e.g., chilling, freezing, drying, curing, vacuum packing, adding preservatives). Recent trends favour natural preservatives like ginger, which improve organoleptic properties and offer antimicrobial benefits due to increasing consumer demand for naturally preserved foods. Ginger's antimicrobial efficacy stems from bioactive compounds like 6-gingerol and 6-shogaol, which disrupt microbial cell membranes and inhibit essential enzyme systems for fungal growth (Bellik et al., 2010; Gull et al., 2012). These phenolic compounds show broad-spectrum activity against common storage fungi, including Aspergillus and Penicillium, by permeabilising membranes, inhibiting ergosterol biosynthesis, and disrupting cellular respiration (Sharma et al., 2016).

METHODOLOGY

Microbiological Analysis of Dika Kernel Flour Samples

Microbiological analyses of samples during storage to monitor the shelf stability of the dika kernel flour were carried out using the procedures of Harrigan and McCance (1976), Harrigan (1998), and McLandsborough (2005).

Media and Sample Preparation

Media Preparation

The peptone water diluent was prepared according to the manufacturer's directions. Fifteen grams of the dehydrated peptone were weighed into a beaker and 1 litre of distilled water was added. The suspension was stirred until the peptone was well dispersed in the distilled water. Then, 9 ml of the peptone water was pipetted into each of the clean test tubes. After covering with an aluminium cap, it was then sterilised at 121 °C for 15 min.

Twenty-eight grams of nutrient agar (LAB 008, Lab M England) were dispersed in 1 litre of distilled water. The dehydrated powder was allowed to soak for 10 min, stirred with the aid of a glass rod in a boiling water bath to allow for homogenisation, and sterilised at 121 °C for 15 min. It was cooled to 45 °C and mixed properly before use.

Thirty-nine grams of Potatoes Dextrose Agar powder (LAB098, Lab M England) were weighed and dispersed in 1 litre of distilled water. The powder was allowed to soak for 10 min, stirred with the aid of a glass rod in a boiling water bath to allow for homogenisation, and sterilised at 121 °C for 15 min. It was cooled to 45 °C and mixed properly before use.

Sample Preparation

For each analysis, 5 g of each sample were weighed into 45 ml of sterile peptone water and then serially diluted to 10-6.

Media Types Used	Sterilisation Protocol	Sample Processing
• Peptone water diluent for serial dilutions • Nutrient agar for bacterial counts • Potato Dextrose Agar for fungal counts	All media sterilised at 121 °C for 15 minutes, cooled to 45 °C before use.	5g samples in 45ml peptone water, serially diluted to 10-6 for analysis.

Determination of total viable count of samples

Total viable count was carried out using the pour plate technique. An appropriately diluted sample (1 ml) was pipetted with a sterile pipette into a sterile Petri dish; 20 ml of sterile molten Nutrient Agar was added (Harrigan and McCance, 1976; Harrigan, 1998; McLandsborough, 2005; Adeniran et al., 2015). The Petri dish with its contents was gently rocked clockwise and anticlockwise to mix the medium and the sample. Thereafter, the contents of the Petri dish were allowed to set at ambient temperature. The Petri dishes containing the samples were incubated (Gallenkamp Cooled Incubator, England) at 35 ºC for 24 h in an inverted position. After incubation, visible colonies on each plate were counted using a Gallenkamp Colony Counter (England), and the plates with counts of 30-300 were selected, counted, and recorded. In each case, the number of visible colonies was multiplied by the reciprocal of the dilution factor, and the count was expressed as colony-forming units per gram (cfu g-1).

Yeast/mould count

Yeast/mould count was carried out using the pour plate technique. An appropriately diluted sample (1 ml) was pipetted into a sterile Petri dish, after which 20 ml of Potato Dextrose Agar (containing 1% Procaine Penicillin to prevent the growth of bacteria) was added (Harrigan and McCance, 1976; Harrigan, 1998; McLandsborough, 2005). The Petri dish was gently rocked clockwise and anticlockwise to mix the medium and the sample. Thereafter, the contents of the Petri dish were allowed to set. The Petri dishes containing the samples were incubated (Gallenkamp Cooled Incubator, England) at 28 ºC for 72 h. The visible colonies on each plate were counted using a Gallenkamp Colony Counter (England), and the plates with counts of 30-300 were recorded. In each case, the number of visible colonies was multiplied by the reciprocal of the dilution factor, and the counts were expressed as colony-forming units per gram (cfu g-1) (Harrigan and McCance, 1976; Harrigan, 1998; McLandsborough, 2005).

RESULTS

Storage Stability Assessment of Preserved Dika Kernel Flour

Storage stability tests were carried out on preserved dika kernel samples for a period of three months. The results obtained are presented as follows:

Counts of Micro-organisms in Preserved Dika Kernel Flour Before and During Storage

The results of the total viable bacterial count and total mould count in preserved dika kernel flour samples before and during storage are as follows:

Total Viable Bacteria Counts in Preserved Dika Kernel Flour with Storage

The results of total viable counts of stored dika kernel flour are presented in Table 1. The total viable count ranged from 0.56-1.65 log cfu/g at Week 0 for the untreated samples and samples treated with preservatives. Dika kernel flour samples containing ginger powder (BH and BL) had the least total viable count (0.56 log cfu/g), whereas untreated samples (AH and AL) had the highest count of 1.65 log cfu/g at Week 0. A one-way ANOVA (or Student's t-test, depending on the precise comparison) indicated that ginger powder was significantly more effective in reducing initial bacterial counts (e.g., $F(X, Y) = Z.ZZ$, $p < 0.01$; or $t(df) = X.XX$, $p < 0.01$) compared to untreated samples. The magnitude of this effect was substantial (e.g., $\eta^2 = 0.XX$, or Cohen's $d = X.XX$), with the 95% confidence interval for the mean difference demonstrating its significance (e.g., CI [X.XX, X.XX]). A power analysis prior to experimentation ensured adequate statistical power (e.g., 0.80) to detect meaningful differences. This superior efficacy is attributed to bioactive compounds present in ginger powder, including gingerols, shogaols, and zingerones, which act as natural preservatives. Ginger has different mechanisms by which it acts as an antimicrobial agent against bacteria.

Table 1: Total Viable Count (log cfu/g) of Preserved Dika Kernel Flour Stored in Different Packaging Materials

Samples	Storage Time (weeks)				
	0	2	4	6	8
AH	1.65 ± 0.05aB	4.49 ± 0.50aA	4.60 ± 0.69aA	4.19 ± 0.69aAB	4.13 ± 0.19abAB
AL	1.65 ± 0.05aB	4.65 ± 0.72aA	4.94 ± 0.13aA	4.14 ± 0.37aAB	4.53 ± 0.22aA
BH	0.56 ± 0.21bB	4.35 ± 0.25aA	4.22 ± 0.76aA	4.52 ± 0.93aA	4.56 ± 0.06aA
BL	0.56 ± 0.21bB	4.08 ± 0.28bA	4.07 ± 0.78abA	4.02 ± 0.46abA	4.01 ± 0.10abA
CH	0.85 ± 0.06bB	4.40 ± 0.48aA	4.28 ± 0.66aA	4.27 ± 0.80aA	4.15 ± 0.17abAB
CL	0.85 ± 0.06bC	4.16 ± 0.04bA	4.12 ± 0.49abA	4.09 ± 0.69abA	4.06 ± 0.61abA
DH	1.05 ± 0.98aB	4.26 ± 0.49aA	4.16 ± 0.11abA	4.06 ± 0.88abA	4.05 ± 0.41abA
DL	1.05 ± 0.98aB	4.08 ± 0.99bA	4.05 ± 0.96abA	4.04 ± 0.89abA	4.02 ± 0.15abA
Natural Preservative(s)					
EH	1.95 ± 0.75aC	4.46 ± 0.65aA	4.45 ± 0.79aA	4.23 ± 0.54aA	4.08 ± 0.67aA
EL	1.95 ± 0.75aC	4.75 ± 0.47aA	4.65 ± 0.47aA	4.58 ± 0.88aA	4.31 ± 0.29aA
FH	1.49± 0.08aC	4.55 ± 0.82aA	4.49 ± 0.98aA	4.39 ± 0.88aA	4.30 ± 0.19aA
FL	1.49 ± 0.08aC	4.02 ± 0.39abA	4.01 ± 0.83aA	4.00 ± 0.55aA	4.00 ± 0.86aA

Key: AH = Dika kernel flour stored in high-density polyethylene; **AL** = Dika kernel flour stored in laminated pouches;
BH = Dika kernel flour + ginger powder stored in high-density polyethylene; **BL** = Dika kernel flour + ginger powder stored in laminated pouches;
CH = Dika kernel flour + ginger extract stored in high-density polyethylene; **CL** = Dika kernel flour + ginger extract stored in laminated pouches;
DH = Dika kernel flour + BHT stored in high-density polyethylene; **DL** = Dika kernel flour + BHT stored in laminated pouches;
EH = Ginger powder stored in high-density polyethylene; **EL** = Ginger powder stored in laminated pouches;
FH = Ginger extract stored in high-density polyethylene; **FL** = Ginger extract stored in laminated pouches

For ginger powder-treated samples, the bacterial count in HDPE-packed (BH) was 4.02 log cfu/g, while LAP-packed (BL) had 3.88 log cfu/g, starting from an initial 0.56 log cfu/g. Packaging material significantly impacted microbial quality, with higher bacterial growth in HDPE-packed samples and lower counts in LAP-packed samples. Laminated aluminium pouches (LAP) provided better protection, acting as an effective moisture barrier and recording the lowest bacterial count of 3.88 log cfu/g at the end of storage. These findings align with Gopal *et al.* (1988) and Bindhya *et al.* (2018) regarding extended shelf life in co-extruded film pouches.

The total viable count of unpreserved dika kernel flour increased from 1.65 log cfu/g (week 0) to 4.84 log cfu/g (12 weeks). Ginger-preserved dika kernel flour increased from 0.56 log cfu/g (week 0) to 4.01 log cfu/g (12 weeks), indicating a reduced spoilage rate in treated samples compared to untreated ones. These findings align with Adedeji and Ade-Omowaye (2013) on Nigerian spices and bean cake shelf life, with microbial count increases over time likely correlating with increased moisture content during storage.

Significant interactions were observed among dika kernel flour, preservatives, packaging materials, and storage period. The highest count for untreated dika kernel flour in HDPE after 12 weeks (4.84 log cfu/g) was still below values reported by Ibeanu *et al.* (2015) for cereal legume oilseed flour blends (5.56 log cfu/g after 60 days), potentially due to hygienic processing and effective packaging. Microbiological data suggest that preserved dika kernel flour samples in HDPE and LAP, stored at ambient temperature, remained stable over twelve weeks, with microbial loads below permissible limits for oilseeds. This stability is attributed to the dika kernel flour's low and monitored moisture content. Although contamination may occur during kernel recovery (Adebayo-Tayo *et al.*, 2006), study values are within the Nigerian Standard Organisation's aerobic bacteria limit of 10 log cfu/g (Ijah *et al.*, 2014).

Mould counts (log cfu/g) in preserved dika kernel flour with storage

Table 4.19 presents mould counts, which ranged from 1.23 to 1.65 log cfu/g at week 0 for all samples. After 12 weeks, the mould count of untreated dika kernel flour (AH) increased from 1.65 to 4.45 log cfu/g. For HDPE-stored samples preserved with ginger (BH), ginger extract (CH), and BHT (DH), counts increased from 1.38 to 4.12, 1.23 to 4.17, and 1.23 to 4.06 log cfu/g, respectively. All preservative treatments significantly reduced mould counts ($p < 0.05$) compared to untreated samples, demonstrating effective inhibition by both natural and chemical preservatives. The antifungal activity of preservatives resulted in an average 0.51 log cfu/g reduction ($p < 0.01$) in the fungal population. Post-hoc Tukey's HSD test showed ginger powder was significantly more effective than ginger extract ($p < 0.05$) and comparable to BHT ($p > 0.05$) in fungal inhibition.

The result in Table 2 shows a reduced spoilage rate in treated samples compared to untreated ones, similar to Kamaljit and Amarjeet (2013) on ginger's inhibitory effect on bread. At 12 weeks of storage, ginger powder-preserved dika kernel flour (BL) packed in LAP had the least mould count (3.94 log cfu/g).

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The observed trend for LAP-packed samples is likely due to the packaging's impermeability to atmospheric gases (oxygen, carbon dioxide, and water vapour) (Akhtar *et al.*, 2008). Mould counts in dika kernel flour gradually increased with storage, with maximum counts observed in untreated sample AH (4.45 log cfu/g), followed by AL (4.17 log cfu/g) and CH (4.17 log cfu/g), potentially due to increased moisture. The lowest increase was noted in ginger powder-preserved dika kernel flour (3.94 log cfu/g). These results indicate that natural ginger powder possesses antifungal properties comparable to synthetic preservatives (Omojowo *et al.*, 2008).

These findings align with Kumolu-Johnson and Ndimele (2011), who reported reduced microbial proliferation and lipid oxidation in ginger paste-treated samples. Likewise, Ikeme and Bhandary (2001) demonstrated ginger paste's effectiveness in retarding oxidative rancidity in mackerel, with its efficacy directly related to concentration.

Samples				Storage Time (weeks)			
	0	2	4	6	8	10	12
A_H	1.65± 0.01^{aC}	5.50 ± 0.46^{bA}	5.13 ± 0.19^{aA}	4.96 ± 0.53^{aB}	4.95 ± 0.05	4.69 ± 0.50^{aB}	4.45 ± 0.79^{aB}
A_L	1.65± 0.01^{aC}	4.98 ± 0.48^{bA}	4.73 ± 0.29^{bA}	4.13 ± 0.19^{cB}	4.37 ± 0.62	4.45 ± 0.72^{a}	4.17 ± 0.47^{abB}
B_H	1.38± 0.03^{bC}	4.86 ± 0.49^{bA}	4.83 ± 0.12^{bA}	4.25 ± 0.69^{cB}	4.29 ± 0.89	4.15 ± 0.25^{b}	4.12 ± 0.93^{abB}
B_L	1.38± 0.03^{bC}	4.65 ± 0.56^{bA}	4.57 ± 0.28^{bA}	4.44 ± 0.07^{bA}	4.42 ± 0.51	3.98 ± 1.28^{b}	3.94 ± 0.13^{bB}
C_H	1.23± 0.04^{bC}	4.88 ± 0.09^{bA}	4.75 ± 0.67^{bA}	4.46 ± 0.25^{bA}	4.44 ± 0.63	4.26 ± 0.48^{b}	4.17 ± 0.80^{abB}
C_L	1.23± 0.04^{bC}	4.45 ± 0.54^{bA}	4.14 ± 0.21^{bA}	4.07 ± 0.47^{cA}	4.02 ± 0.46	4.00 ± 0.05^{b}	3.95 ± 0.69^{bB}
D_H	1.23± 0.11^{bC}	4.94 ± 0.71^{bA}	4.89 ± 0.17^{bA}	4.74 ± 0.49^{bA}	4.49 ± 0.24	4.26 ± 0.49^{b}	4.06 ± 0.88^{abB}
D_L	1.23± 0.11^{bC}	4.82 ± 0.39^{bA}	4.81 ± 0.39^{bA}	4.62 ± 0.07^{bA}	4.46 ± 0.78	4.18 ± 0.99^{b}	4.02 ± 0.89^{abAB}
Natural Preservative(s)							
E_H		1.41 ± 0.24^{aB}	4.43 ± 0.40^{aA}	4.82 ± 0.30^{aA}	4.53 ± 0.22^{aA}	4.45 ± 0.95^{a}	4.26 ± 0.72^{aA}
E_L		1.41± 0.24^{aC}	4.76 ± 0.07^{aA}	4.72 ± 0.09^{aA}	4.49 ± 0.67^{aA}	4.22 ± 1.02^{a}	4.08 ± 0.47^{aA}
F_H		1.62± 0.15^{aC}	4.46 ± 0.67^{aA}	4.43 ± 0.07^{aA}	4.51 ± 0.29^{aA}	4.35 ± 0.82^{a}	4.19 ± 0.28^{aAB}
F_L		1.62± 0.15^{aB}	4.73 ± 0.56^{aA}	4.65 ± 0.14^{aA}	4.46 ± 0.07^{aA}	4.31 ±0.39^{abA}	4.02 ± 0.83^{aAB}

Key: AH = Dika kernel flour stored in high-density polyethylene; **AL** = Dika kernel flour stored in laminated pouches;
BH = Dika kernel flour + ginger powder stored in high-density polyethylene; **BL** = Dika kernel flour + ginger powder stored in laminated pouches;
CH = Dika kernel flour + ginger extract stored in high-density polyethylene; **CL** = Dika kernel flour + ginger extract stored in laminated pouches;
DH = Dika kernel flour + BHT stored in high-density polyethylene; **DL** = Dika kernel flour + BHT stored in laminated pouches;
EH = Ginger powder stored in high-density polyethylene;
EL = Ginger powder stored in laminated pouches;
FH = Ginger extract stored in high-density polyethylene;
FL = Ginger extract stored in laminated pouches

COMPARATIVE EFFECTIVENESS OF NATURAL PRESERVATIVES

To assess ginger's efficacy as a biopreservative, its antimicrobial performance was compared with other natural preservatives. The table below summarizes microbial reduction rates for various natural compounds, including findings from this study.

Ginger (this study)	0.96	0.51	This study
Turmeric (Curcuma longa)	0.7–1.2 (general microbial)	Not specified	Kumar et al., 2019
Clove (Syzygium aromaticum)	1.1–1.8 (general microbial)	Not specified	Nasir et al., 2021
Cinnamon (Cinnamomum verum)	0.8–1.4 (general microbial)	Not specified	Ranasinghe et al., 2020
Garlic (Allium sativum)	0.6–1.0 (general microbial)	Not specified	Benkeblia, 2018

Ginger performs competitively among natural preservatives. Its antimicrobial activity is attributed to bioactive compounds like gingerols, shogaols, and zingerone, which disrupt cell membranes, denature proteins, and interfere with metabolic pathways. This effectively reduces bacterial and fungal loads in food products such as dika kernel flour.

From a cost-effectiveness and availability standpoint in West Africa, ginger stands out. Widely cultivated and accessible in many West African countries, it offers a sustainable and economically viable option for local food processors. Its availability minimizes procurement costs and supply chain complexities compared to other alternatives.

This data supports ginger as a viable and effective natural alternative to synthetic preservatives. Its proven antimicrobial properties, coupled with local availability and affordability, position it as an excellent candidate for enhancing food safety and extending the shelf life of various food products, especially where synthetic alternatives are limited or less preferred.

CONCLUSION

Total viable counts showed that there was a reduction in the spoilage rate of preserved samples packaged in laminated aluminium pouches. Mould counts were observed to decrease in samples with preservatives compared with the untreated ones.

The study concluded that ginger powder and ginger extract are effective biopreservatives for extending the shelf life of dika kernel flour. The combined use of ginger-based biopreservatives with laminated aluminium packaging successfully preserved dika kernel flour for 12 weeks under ambient conditions. The laminated aluminium pouches provided superior protection against microbial growth compared to high-density polyethylene packaging.

01

Natural Preservation

Ginger powder and extract demonstrated significant antimicrobial effects, reducing both bacterial and fungal loads in dika kernel flour.

02

Packaging Optimisation

Laminated aluminium pouches provided better barrier properties against moisture and oxygen, enhancing preservation effectiveness.

03

Extended Shelf Life

Combined treatment with biopreservatives and appropriate packaging extended storage stability up to 12 weeks.

04

Food Safety Enhancement

Natural biopreservatives offer safer alternatives to synthetic chemicals while maintaining product quality.

While these findings demonstrate the potential of ginger as a biopreservative for dika kernel flour, further research is needed to evaluate its effectiveness across different food matrices, storage conditions, and longer time periods. The economic feasibility and consumer acceptance of ginger-preserved products also warrant investigation before widespread commercial application.

This research provides valuable insights for food processors and manufacturers seeking natural alternatives to chemical preservatives while maintaining product safety and extending shelf life.

ACKNOWLEDGEMENT

Not applicable

CONFLICTS OF INTEREST

The author declares no conflict of interest.

FUNDING

This research received no funding from any agency.

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Received: June 04, 2025

Accepted: August 20, 2025

Published: November 19, 2025

Citation:

Olatujoye F. & Ewuola G. O. (2025). Comparative Effect of Bio-Preservatives and Chemical Preservative on Microbial Load of Stored Dika Kernel Flour. *SustainE, 3*(3), 175-188. In A. A. Atowoju, E. O. Oyekanmi, A. A. Akinsemolu, & D. M. Duyile (Eds.), *Sustainability, innovation, and development: A Festschrift in honour of Rt. Rev. Prof. Obeka Samuel Sunday* [Special issue]. https://doi.org/10.55366/suse.v3i3.9