International Journal of
Food Sciences
(IJF)

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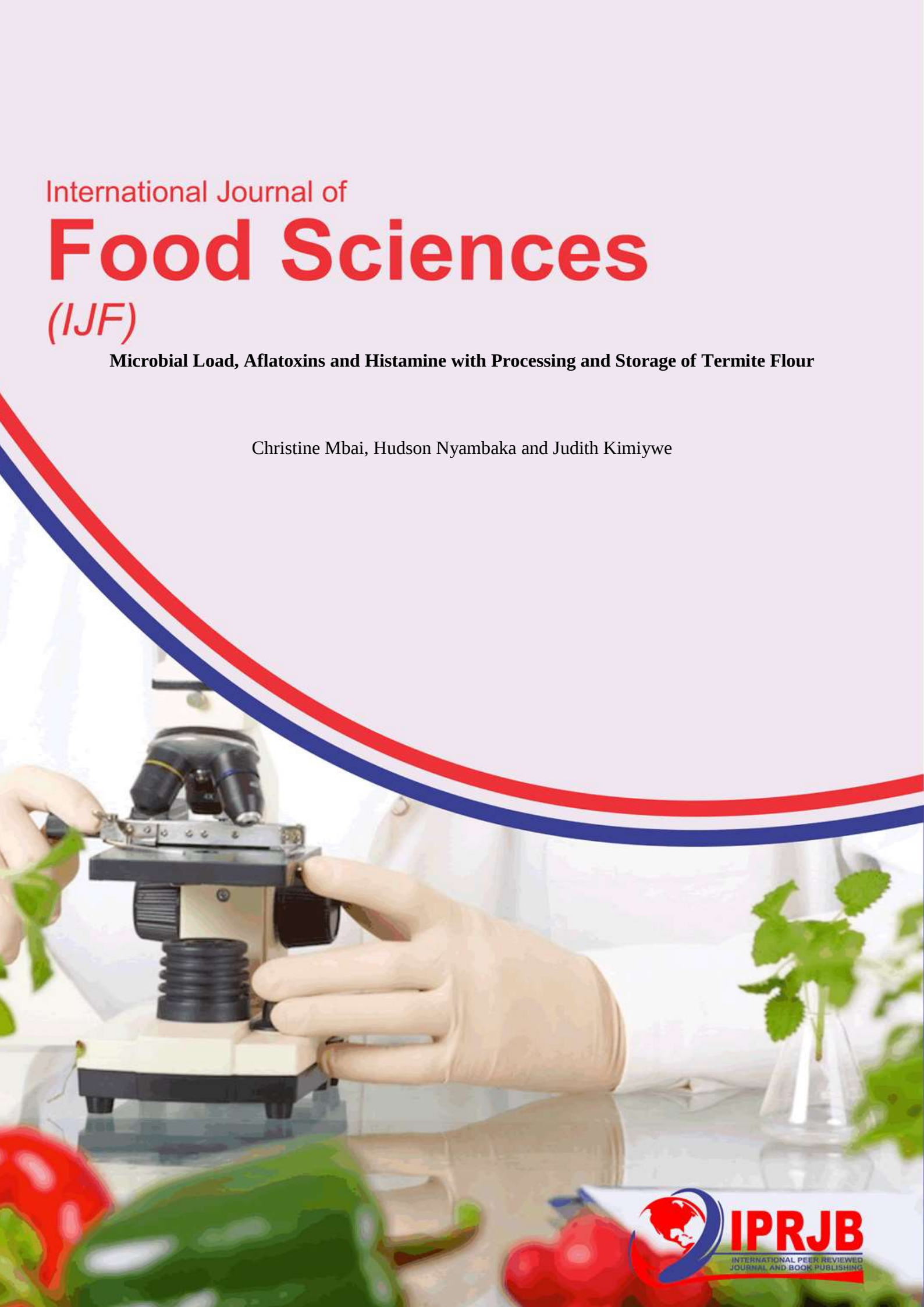

IPRJB
INTERNATIONAL PEER REVIEWED JOURNAL AND BOOK PUBLISHING

International Journal of Food Sciences
ISSN 2789-7680 (online)
Vol. 9, Issue 1, No. 1, pp 1 – 21, 2026

www.iprjb.org

Microbial Load, Aflatoxins and Histamine with Processing and Storage of Termite Flour

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Article History

Received 5th April 2026

Received in Revised Form 7th May 2026

Accepted 3rd June 2026

How to cite in APA format:

Mbai, C., Nyambaka, H., & Kimiywe, J. (2026). Microbial Load, Aflatoxins and Histamine with Processing and Storage of Termite Flour. *International Journal of Food Sciences*, *9*(1), 1–21. https://doi.org/10.47604/ijf.3792

Abstract

Purpose: The current population growth directly translates to an increased strain on the existing food resources. The increasing population needs a sustainable supply of food, especially proteins. However, given that much of the protein required is sourced from livestock that is straining on the environment, unconventional protein sources that are less strenuous, like edible insects, need to be sought. In many regions of the globe, consumable insects have attained more attention as a viable animal protein source. The majority of edible insects are gathered from their natural habitats, although semi-taming and enclosed farming is gaining popularity. However, when stored, insects and processed insect foods like flour may develop aflatoxins and microbial growth. This study assessed microbial load, aflatoxin B1, and histamine levels in flours of termites found in western Kenya.

Methodology: The assessment was through laboratory procedures of the levels as a function of processing and time of storage for three months. Determination of microbial load was done by incubating the samples at room temperature. The yeast plates were removed after twenty-four hours, and mold and bacteria plates removed after seventy-two hours after which colonies were counted. The extraction of aflatoxins involved using methanol mixed with small amounts of water (seventy percent methanol). Histamine was determined using an enzyme-linked immunosorbent (ELISA), a protein-based protocol.

Findings: The study found processing to eliminate histamine in termites. Aflatoxins levels were high in oven-dried samples. Microbial loads were not detected in any of the samples refrigerated at 3˚C, whereas for the bench samples, oven drying was found to eliminate bacteria, yeasts, and molds in whole termites. All insect samples exhibited statistically significant differences ($p<0.0001$), implying that processing impacted histamine, aflatoxin, and microbial load levels. The termite samples had significant differences ($Sig < 0.001$) in the concentration of aflatoxin in the storage period. There was a sharp rise in aflatoxin concentration in most samples within the first 30 days of storage, indicating ideal conditions for fungal growth in this period. Mold counts declined with time in room-temperature samples, while refrigerated samples started manifesting at day 30 and decreased through to the 90th day. Bacterial counts for both the bench and refrigerated insect samples fluctuated throughout the storage duration but were found to be within the legislated limits for minced meat. Yeasts under bench condition displayed a decline in the first 60 days, followed by a pronounced increase towards the end of the 90-day storage duration, whereas refrigerated samples showed increasing yeast levels. Processing and storage affect histamine, aflatoxin, and microbial loads, affecting their growth, survival, and overall stability. Oven dried samples indicated lowest levels of infection for most of the parameters under the study. Refrigerated samples were least infected by microbes.

Unique Contribution to Theory, Practice and Policy: Optimizing processing and preservation methods is vital for maintaining food safety, extending shelf-life, and ensuring product quality to protect consumer health.

Keywords: *Termites Store, Oven Drying, Sun Drying, Microbial Load, Histamine, Aflatoxins*

JEL Codes: *Q18, Q57, I15, L66, O13*

INTRODUCTION

The current global population is causing strain on the existing world resources, especially the scarcity of food. Food scarcity leads to malnutrition, diseases, and even death (McGuire, 2015). A World Health Organization (WHO) report (2024) estimates that one in ten people fall ill from unsafe food each year worldwide. The world's population today, estimated at 7.6 billion is projected to reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion in 2100, according to a 2020 United Nations Report. Likewise, Africa keeps experiencing high population growth rates, with the population of 26 African countries being expected to double up between 2017 and 2050. Thus, producing cheap and sufficient food safe for consumption is a major concern in reducing malnutrition-related deaths (Charlton, 2016).

The risk of micronutrient shortage is boundless in Kenya's chiefly plant-based complementary diet (Kinyuru *et al*., 2015). Consequently, edible insects have been identified as a novel source of food for nutrition diversification to provide adequate nutrient intake in complementary feeding (Dewey and Vitta, 2013; Kelemu *et al*., 2015), and to curb malnutrition in kids (Conti *et al*., 2021). Insects are a good source of proteins, vitamins, vital fatty acids, and micronutrients (Jankowski *et al*., 2025), and their nutritional profile is similar to that of commonly reared livestock (Kamau et al. 2018). Additionally, they are cheap to manage, need less space and water for rearing, and have no major negative effects on the ecosystem. Insects are also eco-friendly because they release fewer greenhouse gases into the atmosphere (van Huis and Oonincx, 2017).

Insects for human food are utilized in many areas of Africa, America, and Asia (Kim *et al*., 2019), and have been used to ease global food shortages. The precise number of types of consumable insects in African countries is unknown. The use of insects as food is intensely subjective to the cultural perceptions as part of what is acceptable to be eaten in contemporary times (Looy, et al., 2014; Hartmann *et al*., 2015; Tan *et al*., 2015). In some nations, some communities have been reported to eat various edible insect species, with neighboring communities abstaining from eating very similar species.

The insects, like lake-flies, grasshoppers, locusts, and termites, are reared in insect manufacturing farms, from egg through their metamorphosis to their mature form (Gahukar, 2016). Some may also be collected from wild habitats. The insects can be eaten whole or processed into other food products, such as bakery products or refreshments, after they are freeze-desiccated and ground into insect powder or packed whole (Lamsal *et al*., 2019). Nevertheless, bulk manufacturing in the insect industry is a concern due to insufficient skills and monies to gather and harvest insects efficiently (Shockley and Dossey, 2014; Aaron et al., 2016; Dossey et al., 2016). The machinery would have to house a suitable enclosure for each life cycle. The production also has to think through the shelf life of insect food products because some can cause concerns for food safety. Careful attention to the product configuration, processing considerations, packing, environmental aspects, biological reactions, chemical changes, and nature of microorganisms present is what testing of shelf life involves (Brown et al., 2011).

Insects are capable of hoarding prospective hazards, such as contaminants, heavy metal concentrations, pathogens, in addition to insecticides (Van Huis, 2013). During long refrigerated storage, various psychotropic bacteria can develop and yield proteases unaffected by heat regulation (*Pseudomonas, Aeromonas, Serratia)*, lipases, as well as spore-forming bacteria. Edible insects have been linked to histamine poisoning, with consumers exhibiting

symptoms such as vomiting, nausea, headache, dyspnea, and bronchospasm (Chomchai & Chomchai, 2018). Fungal classes like *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* produce aflatoxins, which are some of the very lethal metabolites resulting from polyketides (Zain, 2011) and can result in severe dangers to anthropological and animal health by triggering several hitches like hepatoxicity, teratogenicity, and immunotoxicity. Microbial risks with edible insects are also a significant safety concern as the insects' sanitary conditions may not be ideal due to a lack of regulation, control, and information (Fernandez-Cassi *et al*., 2018).

Termites, particularly *Macrotermes* species, are widely consumed in western Kenya and are culturally significant as a protein source. They are known to contain relatively high levels of free histidine, which can be converted into histamine during improper storage or microbial activity. Histamine accumulation has been linked to food poisoning symptoms such as nausea, vomiting, and bronchospasm (Chomchai & Chomchai, 2018). This biochemical profile makes termites a suitable candidate for assessing histamine risks in edible insect flours.

It is difficult to achieve microbiologically hazard-free insects' post-harvest. Insects and insect flour are easily spoiled by a variety of microbes. Aflatoxin and histamine have also been suspected to poison insect food during storage. The contamination of edible insects with unwanted microorganisms is due to a combination of farming or collection methods, substrate, the insect species, and the processing steps involved (Nyangena *et al*., 2020). Formulation of poisonous compounds, gas production, and the formation of slime, in addition to off-flavour production (Rawat, 2015), are some of the consequences of these contaminations due to microbial growth. This may lead to a reduction in the quality and shelf life of insects and insect food. For these reasons, it is essential to improve and standardize methods of processing insects so that the nutritional value and safety of edible insects and insect-based foods can be guaranteed.

Drying is a critical preservation method because it reduces moisture content and water activity (a_w), thereby inhibiting microbial growth and mycotoxin production. Most spoilage organisms and toxin-producing fungi require $a_w > 0.70$for growth, while aflatoxin production is effectively suppressed below $a_w = 0.60$(Brown et al, 2011). Evaluating drying methods such as oven drying and sun drying, alongside storage conditions, is therefore essential to determine whether termite flours can be stabilized for safe consumption. Some popular methods of processing edible insects used in Africa include drying, boiling, steaming, freezing, smoking, frying, and sometimes, in combination (Melgar-Lalanne et al., 2019; Obopile & Seeletso, 2013;). Evaluating and validating these methods would be a robust entry point for the development and implementation of a successful food safety mechanism. Thus, the present work aimed to assess the effect of some of these popular processing methods - sun drying and oven drying, as well as storage time on aflatoxin, microbial load, and histamine levels in termites' insect flour.

Although edible insects are praised for their eco-friendly attributes requiring minimal land, water, and feed inputs, the same factors often introduce food safety hazards. Wild collection exposes insects to environmental contaminants, while low-input rearing systems lack standardized hygiene protocols. These conditions increase the likelihood of microbial contamination, heavy metal accumulation, and mycotoxin development, underscoring the paradox between sustainability and food safety (Van Huis, 2013; Fernandez-Cassi et al., 2018). This paradox highlights the need for empirical studies that balance nutritional promise with safety assurance, leading directly to the problem under investigation.

Statement of the Problem

Globally, the FAO projects that arable land per person will shrink to 0.15 hectares by 2050, while WHO reports that one in ten people fall ill annually from unsafe food, underscoring the urgency of safe, sustainable protein alternatives (FAO, 2023; WHO, 2024). Edible insects have been recognized as viable sources of protein and micronutrients, requiring fewer resources to rear compared to livestock. In Africa, termites and other insects are traditionally consumed, yet their role in modern food systems is constrained by cultural perceptions and safety concerns (Tanga & Kababu, 2023).

In Kenya, termites (*Macrotermes* spp.) are culturally significant and widely consumed in western regions, valued for their taste and nutritional richness. However, recent studies show declining consumption among younger and urban populations, with insects often stigmatized as "emergency food" despite their nutritional potential (Owidi et al., 2025). At the same time, empirical evidence highlights that traditional processing and storage expose insect flours to microbial contamination and aflatoxin risks, undermining consumer confidence (Nyangena et al., 2020; Kinyuru & Ndung'u, 2024). Despite this, little is known about how specific preservation methods such as oven drying, sun drying, and refrigeration affect microbial load, aflatoxin B1, and histamine levels in termites. Addressing this gap is critical to safeguarding consumer health and strengthening the role of edible insects in Kenya's food security agenda**.**

Theoretical Framework

Food Safety / Risk Analysis Framework

This study was guided by the Food Safety / Risk Analysis Framework, articulated through Codex Alimentarius texts developed jointly by FAO and WHO. The framework structures food safety management into three interconnected components: risk assessment, risk management, and risk communication (Codex Alimentarius Commission, 1999; Codex Alimentarius Commission, 2003; WHO, 2024). Risk assessment involves identifying and characterizing hazards such as microbial contamination, aflatoxin accumulation, and histamine formation. Risk management refers to the application of interventions to mitigate these hazards, including processing and storage techniques. Risk communication ensures that consumers, regulators, and producers understand the safety of food products and the measures taken to protect public health.

In practice, the framework has been widely applied in food industries and regulatory systems worldwide. It guides governments in setting safety standards, informs hazard control in food processing, and supports consumer confidence in novel foods. In Africa, it has been used to evaluate emerging insect-based food chains, where microbial and toxin risks are a major barrier to adoption (Tanga & Kababu, 2023). In Kenya, empirical studies show that traditional processing methods expose insect flours to microbial contamination and aflatoxin risks (Nyangena et al., 2020), yet systematic evaluation of preservation methods remains limited. The strength of the framework lies in its systematic, science-based approach to hazard identification and control, integrating technical interventions with consumer protection (FAO, 2023).

Nonetheless, critics highlight limitations in applying the framework in low-resource settings. Laboratory capacity, regulatory enforcement, and risk communication are often weak, especially in informal food chains. This makes implementation challenging in rural communities where edible insects are most consumed. Despite these limitations, the framework is highly relevant to the current study. By testing oven drying, sun drying, and refrigeration,

the study directly engages with risk management strategies, while measuring microbial load, aflatoxin B1, and histamine levels as hazard outcomes. The independent variables (processing and storage methods) represent interventions within risk management, while the dependent variables (safety outcomes) provide evidence for risk assessment. In this way, the Food Safety / Risk Analysis Framework provides the methodological backbone of the study, ensuring that findings contribute not only to academic knowledge but also to policy and consumer confidence in edible insects.

METHODOLOGY

Sample Collection and Preparation

The termite samples were collected from Western Kenya Bondo in Siaya county during harvest, packed in airtight polythene bags, transported to Kenyatta University, and stored in a freezer within twelve hours after collection. Some insects were later sun-dried and oven-dried. The sun drying was done at a normal temperature of 25 to 38 degrees Celsius for 5 days, 7 hours each, while oven drying was done at 60 degrees Celsius for 48 hours. Moisture content analysis showed that fresh insect samples had 40-56%, which was reduced to 9-10% with sun-drying, and 4-5% with oven-drying. The dried samples were pulverized using a blender to obtain fine flour. The fresh samples were made into flour by use of a manual motor and a pestle because of moisture.

Analysis

Microbial load, aflatoxins, and histamine analysis was done following the Association of Official Agricultural Chemists (2006) methods. Aflatoxins and histamine were reported in ppb while microbial load was reported in CFU/g.

Histamine Analysis of Samples

Enzyme-linked immunosorbent (ELISA), a protein-based protocol, was used. The specific enzyme-labeled antibody was then used for binding. The estimation for the resulting antigen/antibody complex concentration was then estimated based on a curve generated with reference standards that are purified in an air-conditioned laboratory.

Aflatoxin Analysis of Samples

The extraction of aflatoxins involved the use of methanol mixed with small amounts of water (seventy percent methanol). The standard essay format took 90 minutes to conduct. The purified antibody was diluted in fifty millimolar carbonate buffer (pH 9.6) to ten micrograms per milliliter. The antibody solution (one hundred microliters) was introduced to each microwell, and the solution was left to incubate overnight. The microwells were cleaned repeatedly for five times with 0.05percent Tween twenty. Following the drying of wells, one hundred microliters of five percent casein in fifty micromolar phosphate buffer containing 0.9 Percent Sodium Chloride (PBS) was placed in each well, and the solution was nurtured for 15 minutes. The test was performed by the addition of one hundred microliters of sample extract and one hundred microliters of enzyme conjugate in 1 percent Fish Hydrolysate (FH) in PBS, and the competitive reaction was allowed to take place for 15 minutes. One hundred microliters (100 mL) of deionized water were contained in the control wells. The food contents were discarded, and microwells washed 5 times with a half Tween 20. Colour was developed by addition of 100microlitre per well enzyme substrate (3.3, 5.5 tetramethylbenzidines in 0.05M sodium acetate buffer, pH 5.5) was added to develop colour, and the reaction sustained for 10 minutes. The addition of a microliter of 1.25M H_2SO_4 to each well stopped the colour reaction

and absorbance read at 450nm using a Multiskan Ascent microplate reader (Lab systems, Finland), whose range was 340nm-850nm. All incubations were carried out in an air-conditioned laboratory at room temp (20-24°C).

Microbial Load Analysis of Samples

Insect samples (thirty grams for every type under treatment) were ground using a universal blender (Caparros Megido *et al*., 2017). Prior to utilization, the blender's grinding compartment was sterilized by autoclaving. To verify the effectiveness of sterilization and aseptic handling, negative controls were included by plating sterile sodium chloride solution (0.85% NaCl) without insect material on Nutrient Agar and Potato Dextrose Agar. These blank agar plates were incubated under the same conditions as the test samples, and no microbial growth was observed. Microbial analysis considered both mesophilic and psychrophilic bacteria, since some species are adapted to grow at low temperatures. This ensured that microbial growth patterns under refrigerated storage could be accurately captured.

The ground powdered samples were stored in two different conditions i.e. bench and refrigerated. The bench samples were placed in clean air-tight containers, on clean shelves in a cupboard at room temperature and analyzed at 0, 30, 60, and 90 days. The refrigerated samples were kept in airtight containers in a fridge at three degrees Celsius and analyzed at the same intervals as the bench samples. There was suspension of ten grams of ground material in a 90-milliliter sterile sodium chloride solution in a one-hundred-milliliter conical flask for one minute. The suspensions underwent 10-fold consecutive dilutions (up to 10^{-8} in isotonic sodium chloride solution 0.85% NaCl). One milliliter of suspension was transferred to two different media-Nutrient Agar (NA) for bacteria isolation and Potato Dextrose Agar (PDA) containing oxytetracycline for yeast and mold. Three plates were incubated at thirty degrees Celsius for twenty-four hours for yeast enumeration, while mold plates were incubated for seventy-two hours at room temperature. After incubation, the yeast and mold colonies were counted. The Nutrient Agar plates were incubated at thirty degrees Celsius for seventy-two hours, after which bacterial colonies were counted.

Statistical Analysis

The collected data were entered in Microsoft Excel spreadsheet and summarised as the mean ± standard deviation. Means of the different concentrations were compared with One-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test for means comparison. Statistical significance was executed at 95% confidence level ($p<0.05$), and the analyzed data presented in tables.

RESULTS AND DISCUSSION

Processing

Table 1: Levels of Histamine, Aflatoxins and Microbial Load in Processed Termite

Sample	Treatment	Analyte levels (Mean ± SE)							
		Histamine (ppb)	Aflatoxin (ppb)	Bacteria (102 cfu)-Bench	Bacteria (102 cfu)-3˚C	Yeast (102 cfu) Bench	Yeast (102 cfu)-3˚C	Moulds(102 cfu)-Bench	Moulds (102 cfu)-3˚C
Defatted termite	Oven-dried	0.00±0.00a	0.417±0.010a	281.00±5.292ac	ND	281.33±6.489a	ND	33.00±1.155a	ND
Defatted termite	Sun-dried	0.00±0.00a	0.352±0.012b	266.00±7.00a	ND	293.00±3.606a	ND	33.00±1.732a	ND
Whole termite	Oven-dried	0.00±0.00a	0.746±0.025c	0.00±0.00b	ND	0.00±0.00b	ND	0.00±0.00b	ND
Whole termite	Sun-dried	0.00±0.00a	0.513±0.006d	291.67±2.333c	ND	276.00±13.796a	ND	31.00±0.577a	ND
Whole termite	Fresh	3.199±0.023b	0.634±0.007e	287.33±3.480c	ND	291.33±4.667a	ND	34.67±2.333a	ND
p-value		<0.0001	<0.0001	<0.0001		<0.0001		<0.0001	

NB: Superscript with different letters in the same row indicate statistically significant differences between values (mean ranks) across the row.
ND-not detected; TNTC-too numerous to count; TFTC-too few to count; Count range 30 -300 cfu per plate. One way ANOVA using Tukey's Test was used.

Histamine with Processing

The levels of histamine in fresh and processed termites are shown in Table 1.

All termite samples recorded no histamine except whole fresh termite, which recorded 3.199±0.023 ppb, indicating that defatting and processing of the termite samples significantly($p<0.0001$) reduced the levels of histamine. Notably, the values in this study were significantly lower than the limit of 200 mg/kg (200,000 ppb) set in Regulation EC No 2073/2057 for histamine in fishery products (Turck *et al*., 2021).

There are limited publications that explicitly show the changes in levels of histamine in insects with the sun-drying and oven-drying processing techniques. However, there are analogous investigations on fish that can offer valuable insights. For example, Hwang *et al*., (2012), while investigating the effect of drying methods on the formation of histamine in dried milkfish, found histamine levels were not detected when hot-air drying at 55˚C and at 67.4±51.1 mg/100g (*10^4 ppb) when sun-drying. Additionally, Minh (2019) found histamine levels in dried Gourami to be 0.52±0.03, 0.57±0.02, 0.61±0.05, and 0.65±0.04 mg/kg (*10^3 ppb) after 1, 2, 3, and 4 days, respectively of sun-drying. Histamine levels for samples oven-dried at 44˚C, 48˚C, 52˚C, 56˚C were 0.48±0.01, 0.43±0.02, 0.41±0.01, 0.37±0.00 mg/kg (*10^3 ppb), respectively. Although the values from both Hwang *et al*., (2012) and Minh (2019) were higher than this study's, they both found oven-drying to be more effective than sun-drying.

In insects, histamine exists as a neurotransmitter. However, despite its prevalence and widespread insect foods consumption, scombroid poisoning from the histamine in insects is quite rare (Chomchai & Chomchai, 2018). This is because the levels present are small and are unlikely to be the source of substantial toxicity. It is therefore postulated that the histamine which causes human poisoning is a de novo result of histidine decarboxylation by bacteria (Chomchai & Chomchai, 2018). Therefore, the reduction/inactivation of histamine-producing bacteria reduces histamine formation. Oven drying has greater efficiency in reducing bacterial contamination over sun drying (Dandadzi *et al*., 2023) and may be preferred in order to lower histamine levels.

Aflatoxins with Processing

The levels of aflatoxins in fresh and processed termites are shown in Table 1.

Processing methods significantly influenced aflatoxin levels($p<0.0001$), with levels ranging from 0.352±0.012 ppb in sun-dried defatted termites to 0.746±0.025 ppb in oven-dried whole termites. Generally, higher levels were observed in oven-dried samples. However, the aflatoxin levels were lower than the 2-ppb limit recommended by the European Union (Oni *et al*., 2025).

Aflatoxins can contaminate insects during processing, harvesting, and storage. The efficiency of drying and speed is important, as prolonged and improper drying may eventually lead to a buildup of aflatoxins. For example, poor processing processes like sun drying insects in open environments can lead to aflatoxin contamination. Although studies on the effect of oven and sun drying are scarce, we can draw from Gowda *et al*., (2007), who found the processing methods to reduce toxin levels in contaminated sheep feed by 57.6% and 83.7%, respectively. This study also corroborates the findings of Gowda *et al*., (2007), showing sun-drying was more effective.

It can also be noted for aflatoxins, the defatted samples recorded lower aflatoxin levels than other samples. For example, defatted sun-dried termites were found to have 0.352 ± 0.012^{a} ppb, while whole sun-dried termites exhibited 0.513 ± 0.006^{a} ppb. A study by Ting *et al*., (2020) that had cited Bircan, C. (2006) mentioned that aflatoxin biosynthesis is achieved through lipid peroxidation (oxidative lipid degradation) when fatty acid hydroperoxides are present. The presence of polyunsaturated fatty acids enhances aflatoxin production and lipid peroxidation. As insects are considerable sources of fat, whose oils contain polyunsaturated fatty acids (Womeni *et al*., 2009), and defatting involves removing edible insect lipids (Kim *et al*., 2022), then this may have reduced aflatoxin proliferation in the insect samples in this study.

Aflatoxins are highly heat resistant, as their decomposing temperatures are higher than 235°C, and thus simple drying cannot decrease their levels significantly. Rough heating at temperatures of 150-200°C can remove a substantial portion of AFB1 when there is high humidity, but it may affect other nutrients in the food (Ndagijimana *et al*., 2020). To this end, precautionary measures must be taken regarding edible insects to avoid losses in quantity and quality. Insects should be fed on clean, safe, and mold-free, substrates. Additionally, drying to reduce moisture content is recommended as early as possible before storing in airtight, moisture-proof packaging.

Microbial Load with Processing

The levels of microbial load in fresh and processed termites are shown in Table 1.

For the termite samples refrigerated at 3°C, there was no microbial load detected (ND). Given that no microbial presence was detected, it appears that processing did not affect the microbial load. Low temperatures, such as those used in refrigeration, are known to inhibit the metabolic activity and proliferation of micro-organisms. In contrast to this study, Klunder *et al.*, 2012 reported high levels of micro-organisms in refrigerated fresh insect samples, but on boiling and then refrigerating, the counts were fairly stable for roughly more than two weeks.

For the bench samples, oven-drying eliminated yeasts, bacteria, and mould in whole termite samples. Oven-dried termites generally registered lower levels of bacteria, yeasts, and moulds compared to those in fresh and sun-dried samples, except for the defatted oven-dried sample which had higher bacterial levels than the defatted sun-dried sample. The microbial loads

among the different insect samples exhibited statistically significant differences ($p<0.001$), suggesting that processing techniques significantly impacted the microbial load.

Insects, similar to other products derived from animals, are rich in moisture and nutrients and contain their gut microflora, providing a medium for the growth of unwanted micro-organisms in certain conditions (Klunder *et al*., 2012; Rumpold & Schluter, 2013; Kooh *et al*., 2019). Additionally, the presence of these microorganisms, like bacteria, yeasts, and moulds may be attributed to the release of microbiota from the insects' intestines during grinding and then dispersed across the entire product (Mmari *et al*., 2017).

Bacteria contamination has been of notable concern because of the possible pathogenic species and their effects on the shelf life of insect products. In this investigation, bacteria were reported in all termite samples except the whole oven-dried sample. This finding is consistent with earlier studies which reported that both farm-reared (Vandeweyer *et al*., 2017a; EFSA Scientific Committee, 2015) and wild-caught (Amadi & Kiin-kabari, 2016; Braide *et al*., 2011) edible insects have been linked to a number of bacterial species. Among them are a few species of bacteria from genera such as Acinetobacter, Erwinia, Clostridium, Pseudomonas, and members of the Enterobacteriaceae family (Garofalo *et al*., 2019; EFSA Scientific Committee, 2015; Murefu *et al*., 2019). Some of these are opportunistic and pathogenic and reduce the insect products' shelf life.

The presence of these bacteria, such as those that are endospore-formers that may survive the common processing techniques adopted for edible insects like deep-frying, boiling, and drying, due to their heat resistance are of major concern (Osimani *et al*., 2017a; Klunder *et al*., 2012; Vandeweyer *et al*., 2017b). Spore-forming bacteria have been detected in processed locusts, house crickets, and edible yellow mealworms (Vandeweyer *et al*., 2020; Osimani *et al*., 2017a), and may remain viable in insect products even after processing. This underscores the difficulty of ensuring microbial safety in insect-based food products, especially due to the presence of heat-resistant bacteria.

Earlier studies have also analyzed bacterial levels with processing in edible insects. For example, Grabowski and Klein (2017) examined the microbial properties of some raw and processed insects obtained from Germany or Netherlands. They found that processed insects generally had much lower bacterial levels than raw and unheated ones, emphasizing the role of processing in reducing microbial contamination. However, they cautioned that inadequate processing could lead to an increase in bacterial counts during storage. Raw edible insects were also found to have higher levels of Enterobacteriaceae by Dandadzi *et al*., (2023), that were reduced with sun drying, and eliminated with oven drying.

Oven and sun drying were found to lower the bacterial loads to acceptable limits, when investigating how efficient the processing techniques were in improving the black soldier fly larvae's bacterial quality as animal feed (Mapiki, 2022). The study noted that oven drying was more effective as it lowered the bacterial counts more, which she attributed to the higher temperatures of oven heating, unlike sun drying, whose temperature was dependent on the day's weather. The difference between the findings in the present research and earlier studies from Dandadzi *et al*., (2023) and Mapiki (2022) is that they conclusively found oven drying to be more effective than sun drying.

Yeasts and moulds have been observed to induce deterioration in food products exhibiting a minimal water activity, including edible insects that have been dried. While yeasts have not

been reported to cause food poisoning, moulds in edible insects are of critical concern due to their potential to produce injurious strains of mycotoxin.

Notably, the mold counts in the insects' samples surpassed the limit stipulated by Regulation 2017/2470, which prescribes a level of ≤100 cfu/g (Marzoli *et al*., 2023). Higher levels of yeasts and moulds in unprocessed edible stinkbugs of 3.24 log cfu/g (approx. $17.38*10^2$ cfu/g) were reported by previous researchers, Dandadzi *et al*., (2023) and, sun- and oven drying resulted in a marked decrease. Sun-dried samples recorded 2.46 ± 0.14^c log cfu/g (approx. 288 cfu/g), while oven-dried samples logged 1.37 ± 0.02^b log cfu/g (approx. 23.44 cfu/g). Although their yeast and mold counts were combined, they were significantly lower than those in this study. This could be ascribed to contamination during processing and storage or the difference in the insect species examined in this study. Similar to this research, the study found oven drying to be more effective than sun drying in reducing the yeast and mold levels of edible stinkbugs. Nyangena *et al*., (2020) also found oven drying to reduce yeast and mold levels in A. domesticus (house cricket), H. illucens (black soldier fly), R. differens (longhorn grasshopper), S. littoralis (African cotton leafworm) compared to levels in fresh samples. Toasting and microwaving were found to eliminate yeast and mold counts, (Nyangena *et al*., 2020, Dandadzi *et al*., 2023), although they were not considered in this study.

Current insect microbiology research is in its early phases, and due to variations in insect species, processing, and techniques of analysis and evaluation, results can occasionally be difficult to compare. Overall, processing techniques result in a reduction in microbial loads or even total elimination of some microbes (Dandadzi *et al*., 2023). Moisture content and water activity are reduced via drying, thus reducing microbial proliferation and enzymatic and chemical reactions, hence increasing the insects' products' shelf-life. According to Vandeweyer *et al*., (2018), the initial microbial levels are reduced with heat exposure, and differences in the microbial loads for each processing method could be attributed to the temperatures exposed. As per Grabowski and Klein, 2017, microbial loads are heavily influenced by insect types, treatment-specific conditions, and patterns.

Storage

Aflatoxins

Table 1: Aflatoxin AFB_1 Levels (ppb) in Termite Insect Flour Kept Different Storage Periods

Sample	Processing treatments	Aflatoxin levels at different durations of storage period (days)				ANOVA test*	
		0	30	60	90	χ^2	sig
Defatted termite	Fresh	0.296 ± 0.004^a	1.003 ± 0.029^b	0.697 ± 0.037^c	1.203 ± 0.032^d	151.021	<0.001
	Oven-dried	0.417 ± 0.010^a	1.197 ± 0.057^b	1.197 ± 0.076^{bc}	0.903 ± 0.066^c	32.408	<0.001
Whole termite	Sun-dried	0.352 ± 0.012^a	1.503 ± 0.066^b	1.100 ± 0.062^c	1.600 ± 0.061^b	130.073	<0.001
	Oven-dried	0.746±0.025	0.797±0.013	1.010±0.150	1.100±0.072	4.546	<0.055
	Sun-dried	0.513 ± 0.006^a	1.003 ± 0.061^b	1.800 ± 0.082^c	1.503 ± 0.04^d	139.968	<0.001

NB: Superscript with different letters in the same row indicate statistically significant differences between values (mean ranks) across the row.
ND-not detected; TNTC-too numerous to count; TFTC-too few to count. A repeated ANOVA was used.

The results for aflatoxin concentration in ppb for the fresh and processed termites with storage were as shown in Table 2.

The findings in this study indicate that the storage duration substantially impacts aflatoxin levels in insect flour (*Sig. < 0.001*). Oven-dried termites with a borderline significance (*Sig. <*

0.055), suggests that this treatment inhibited further toxic production through lowered water activity, despite the mean value rising from 0.746±0.025 to 1.100±0.072 ppb. The significant findings highlight the importance of employing stringent storage measures to control aflatoxin levels. For example, the sharp increases at day 30 indicate that this period is critical for fungal activity, emphasizing the necessity for proactive measures such as controlling temperature and moisture.

To the author's knowledge, aflatoxin concentrations in these insect samples over a 90-day storage period have been inadequately evaluated. However, in their work, Kachapulula *et al.,* (2018), found aflatoxin levels in caterpillar to be unsafe when subjected to simulated poor storage. On the other hand, Kinyuru *et al.,* (2015), while investigating the stability of complementary food enriched with termites and fish did not report any aflatoxin over a 6-month storage period. Although the levels detected in this study are lower than the maximum recommended limits, 20 ppb (20 ng/g; World Health Organization), they still highlight the importance of proper storage to avoid potential aflatoxin contamination over time. Musundire *et al*., (2016) also corroborated that proper storage eliminated/reduced aflatoxin contamination.

Microbial Load Levels

Mould Count at Bench and Refrigerated Conditions

Table 3: Moulds (cfu/g) in Termite Samples Kept at Bench and Fridge Conditions between Different Periods of Storage

Sample	Treatment	Condition	Colony count (Mean±SD) at various durations of storage				Friedman test	
			0 days	30 days	60 days	90 days	χ^2	sig
Defatted termite	Oven-dried	Bench	33.00±2.000^{a}	TFTC^{ab}	0.00±0.000^{b}	0.00±0.000^{b}	9.000	0.029
	Oven-dried	Fridge	ND	0.00±0.000	0.00±0.000	0.00±0.000	0.000	1.000
	Sun-dried	Bench	33.00±3.000^{a}	TFTC^{ab}	0.00±0.000^{b}	0.00±0.000^{b}	9.000	0.029
	Sun-dried	Fridge	ND	TFTC	11.67±2.890	0.00±0.000	6.000	0.050
Whole termite	Oven-dried	Bench	0.00±0.000^{a}	TFTC^{b}	0.00±0.000^{a}	0.00±0.000^{a}	9.000	0.029
	Oven-dried	Fridge	ND	TFTC	0.00±0.000	0.00±0.000	6.000	0.050
	Sun-dried	Bench	31.00±1.000^{a}	TFTC^{ab}	11.33±3.220^{ab}	0.00±0.000^{b}	9.000	0.029
	Sun-dried	Fridge	ND	10.00±3.610	7.67±2.520	18.67±1.160	0.000	1.000
	Fresh	Bench	34.67±4.04^{a}	9.67±2.52^{ab}	0.00±0.00^{b}	0.00±0.00^{b}	9.000	0.029
	Fresh	Fridge	ND	TFTC	0.00±0.000	18.67±1.160	6.000	0.050

NB: Superscript with different letters in the same row indicate statistically significant differences between values (mean ranks) across the row
ND-not detected; TNTC-too numerous to count; TFTC-too few to count; Count range 30 -300 cfu per plate.
Posthoc Test using Bonferroni correction was used

Table 3 shows the analysis for moulds with storage in the fresh, sun-dried and oven- dried termites for both bench and refrigerated conditions.

Most termite samples showed a decline in mold scores with time, with the highest values observed at day 0 and the lowest at day 90. This tendency implies that early mold growth is more pronounced in freshly processed samples and subsequently declines with storage, possibly due to reduced oxygen availability over time. As Rawat (2015) noted, moulds require oxygen for their metabolic processes. With storage limiting the oxygen levels, mold proliferation is inhibited, likely accounting for the mold counts observed.

The majority of earlier studies, such as those by Kamau *et al.,* (2018) have combined yeast and mold counts, which may not have adequately captured the behavior of each microbe. In fact, Kamau *et al.,* (2018), did not detect yeast and mold counts in their adult cricket meal on day zero in ambient storage, but they did find that they tended to increase with time in contrast to this investigation. Kouřil *et al.,* (2022) also found moulds in their study of the mealworm in some of their samples stored at ambient conditions. The results in the table show that no mold

counts were observed at day zero across all insect samples stored at 3˚C, indicating minimal initial contamination. However, mold counts were recorded from day 30 and fluctuated through today 90. These results align with Kamau *et al.*, (2018), who reported that mold counts in house cricket meal in refrigerated samples at the beginning of storage were not detected.

While most moulds prefer warmer temperatures, some can grow in refrigerators, too (Rawat, 2015). The mold counts in the samples at 3˚C were lower than those at bench conditions, highlighting that low temperatures inhibit microbial metabolism. On the other hand, bench conditions are more favorable for mold proliferation due to ambient temperatures. However, Kouřil *et al.*, (2022) recommended storing insect powders at bench conditions rather than refrigeration due to a high incidence of mold growth caused by humidity in his study.

Bacterial Load at Bench and Refrigerated Conditions

Table 4: Bacterial Load (Cfu/G) Between Termite Samples Kept at Bench and Fridge Conditions at Different Points of the Storage Period

Sample	Treatment	Condition	Colony count (Mean ±SD) at various durations of storage				Friedman test	
			0 days	30 days	60 days	90days	χ^2	sig
Defatted termite	Oven-dried	Bench	281.00±9.165	161.67±10.214	180.33±10.504	124.67±13.317	0.000	1.000
	Oven-dried	Fridge	ND	137.67±9.018	102.67±17.156	139.67±7.506	0.000	1.000
	Sun-dried	Bench	266.00±12.124	179.00±8.185	203.00±58.592	46.33±6.807	0.000	1.000
	Sun-dried	Fridge	ND	176.33±4.041	217.00±20.421	285.67±6.506	0.000	1.000
Whole termite	Oven-dried	Bench	0.00 ± 0.000^{a}	$TFTC^{ab}$	85.00 ± 7.000^{b}	0.00 ± 0.000^{a}	9.000	0.029
	Oven-dried	Fridge	ND^{b}	25.67 ± 1.528^{ab}	71.33 ± 7.024^{a}	0.00 ± 0.000^{b}	6.000	0.048
	Sun-dried	Bench	291.67±4.041	71.33±9.866	133.67±17.156	230.67±17.926	0.000	1.000
	Sun-dried	Fridge	ND	136.33±29.939	140.67±11.015	161.33±11.015	0.000	1.000
	Fresh	Bench	287.33±6.028	209.00±22.869	198.00±5.568	78.00±24.759	0.000	1.000
	Fresh	Fridge	ND	TNTC	191.33±23.692	246.00±8.888	6.000	0.050

NB: Superscript with different letters in the same row indicate statistically significant differences between values (mean ranks) across the row

ND-not detected; TNTC-too numerous to count; TFTC-too few to count; Count range 30 -300 cfu per plate.

Table 4 shows the analysis for bacterial load with storage in the fresh, sun-dried and oven-dried termites for both bench and refrigerated conditions.

Termite samples displayed no significant change in the bacteria levels over the 90 days, with the notable exception for whole oven-dried termites that exhibited fluctuating loads. The bacterial loads at bench had a general trajectory, with the samples such as whole fresh and sun-dried termites displaying a massive initial surge (at 0 days), followed by a decline toward day 90 as nutrients were exhausted. While these findings may not correlate directly with earlier investigations due to the insect types examined and the storage periods, meaningful insights can still be drawn through comparisons. Machona *et al*., (2024) reported that mealworm (*Tenebrio molitor*) larvae had bacterial counts ranging from 14.79 to 602.56 cfu/g, which were lower than the legislated bacteria in raw material for minced meat or meat preparation. The recommended threshold value for minced meat is 6.70 log cfu/g (Trabelsi *et al*., 2019). The values in this research were within these limits and did not present a threat for bacterial food contamination.

Bacterial growth in refrigerated conditions is attributable to some bacteria species that are adapted for growth at low temperatures (psychrophilic) (Rawat, 2015). The insect samples stored at 3˚C displayed a steady climb in bacterial count over the three months of storage, yet they were not detected at zero day of storage which was indicative of psychrophilic species. Many samples lacked substantial variations (*Sig. =1.000*), indicating that any changes in the

bacterial load were independent of the storage duration. In contrast, oven-dried whole termites (*Sig. = 0.048*) showed a measurable response to storage. For instance, oven-dried whole termites recorded a peak bacterial load at day 60 and a decline by day 90. Bacterial loads were not detected in any insect samples at day 0.

While refrigeration slows bacterial growth, factors possibly related to initial contamination or the sample's inherent properties may contribute to variations over time. The scholarly work on bacterial growth in the insect samples investigated in this study throughout the 90-day storage period is scarce. However, overall patterns stress the importance of refrigeration in suppressing bacterial proliferation compared to ambient conditions. Refrigeration temperatures of 4-6^{0}C. have been reported to increase shelf-life for several days to a couple of weeks (Liceaga, 2021). For instance, Klunder *et al*., (2012), in their investigation of boiled house crickets, reported that those at room temperature storage spoiled rapidly, while the bacterial levels in the refrigerated samples remained fairly stable over a two-week storage duration. To further increase the shelf-life of insects and insect flours, freezing is recommended as it allows for a lower water activity (Liceaga, 2021). This has been depicted in a research by De Smet *et al*., (2019), whereby bacteria causing spoilage persisted in mealworm paste kept at 4^{0}C, reaching levels considered indicative of spoilage, whereas that stored at -21^{0}C did not have an increase in microbial counts for up to three months.

Yeast Counts at Bench and Refrigerated Conditions

Results for yeast kept under bench and fridge (3^{0}C) conditions for 90 days in cfu/g were analyzed and recorded in Table 5 below.

Table 5. Yeasts (Cfu/G) Between Termite Samples Kept Under Bench and Fridge Conditions at Different Points of Storage Period

Sample	Treatment	Condition	Colony count (Mean cfu ±SD) at various points during storage				Friedman test	
			24 hours	30 days	60 days	90 days	χ^2	sig
Defatted termite	Oven-dried	Bench	281.33±11.240	227.33±2.520	131.33±9.070	267.33±12.550	0.000	1.000
	Oven-dried	Fridge	ND	148.67±10.600	139.33±19.01	TNTC	6.000	0.050
	Sun-dried	Bench	293.00±6.240	270.00±10.000	236.67±45.090	287.67±7.020	0.000	1.000
	Sun-dried	Fridge	ND	149.33±9.02	133.33±15.28	TNTC	6.000	0.050
Whole termite	Oven-dried	Bench	0.00±0.000^a	70.67±27.010^b	46.00±12.170^b	150.33±16.560^b	9.000	0.029
	Oven-dried	Fridge	ND^a	TFTC	TFTC	10.67±8.960	0.000	1.000
	Sun-dried	Bench	276.00±23.900	110.67±9.010	104.33±16.040	286.33±7.020	0.000	1.000
	Sun-dried	Fridge	ND^b	0.00±0.00^a	TFTC^{ab}	TNTC^b	6.000	0.049
	Fresh	Bench	291.33±8.080^a	210.00±13.750^a	176.67±23.180^a	TNTC^b	9.000	0.029
	Fresh	Fridge	ND^a	287.67±5.510	28.00±2.650	TNTC	5.636	0.060

NB: Superscript with different letters in the same row indicate statistically significant differences between values (mean ranks) across the row

ND-not detected; TNTC-too numerous to count; TFTC-too few to count; Count range 30 -300 cfu per plate.

Samples under bench condition exhibited a highly volatile trend, where yeast populations reduced at 30 and 60 days, followed by a sharp resurgence at 90 days. However, in some cases, such as with whole termite (sun-dried), there was no statistical significance (*Sig. = 1.000*). Kouřil *et al*., (2022) investigated the effect of several storage techniques on the microbial quality of dry mealworm (*Tenebrio molitor*) insect powder for 8 months. Yeasts were not detected for the entire period for samples stored at 22-23˚C. These findings contrast with the values in this study, as yeasts were present in all samples.

According to the established Germany and European community threshold, yeast levels should lie in the range of 1-3 cfu/g (Grabowski and Klein, 2017). However, this study's findings were not within this range, raising concerns about potential safety hazards and the need for further research to guarantee that the product satisfies acceptable health standards. The prevalence of the yeast count can be due to contamination during processing.

Yeast counts in refrigerated samples were initially undetected in all insect samples after 0 days, but storage conditions affected their presence with time, revealing varying patterns across all samples. Storage had an impact on most termite samples, like defatted termites, where yeast proliferation fluctuated, with levels decreasing by day 60 and then culminating in TNTC (Too Numerous To Count) at the 90th day (*Sig. 0.050*), further supporting the significance of the storage duration.

Kouřil *et al*., (2022) did not detect yeast counts in their work even after 8 months in insect samples stored in the refrigerator at 4-6˚C, unlike in this study, where yeasts were present from day 30 to day 90. Kamau *et al*., (2018) also did not detect any yeasts at day zero in their investigation of the effect of storage temperature, duration, and packaging material on semi-processed adult house cricket meal quality over 180 days, even though yeasts and moulds were reported as a combined group. They also stated that the yeast counts increased with time, although the levels were lower than in ambient conditions. Variations in the findings of this study may be attributed to the depletion of nutrients in samples whose yeast counts were reduced and contamination in samples where the yeast count prevailed.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Processing

Histamine reduced significantly with processing for most samples, where the highest concentration was for whole fresh termite, and none recorded in all the other termite samples. Processing significantly affected the aflatoxin level. The lowest concentration was in defatted sun-dried termites, and the highest concentration was in whole oven-dried termites (p-value < 0.001). Although oven-drying correlated with higher aflatoxin levels in whole termites, these values remained well below international safety limits. Oven-drying was considered superior because it consistently eliminated bacteria, yeasts, and molds in bench-stored samples, achieving microbial safety through higher and more stable drying temperatures. This microbial elimination outweighs the modest aflatoxin increase, making oven-drying the most effective technique overall. For the refrigerated samples, no microbial load was detected for all samples, indicating that processing did not significantly impact the concentration of the microbial load on the first day.

Storage

Storage significantly affected aflatoxin in the termite samples (*Sig. < 0.001*), with a sharp increase in the first 30 days. Refrigerated conditions initially suppressed microbial loads, but the rise in yeasts and psychrophilic bacteria (cold-adapted species capable of growing at low temperatures, such as members of *Pseudomonas* and *Enterobacteriaceae*) over the 90 days indicates that low temperatures only delay spoilage. Under bench condition, yeast levels decreased at 30 and 60 days, before increasing by the end of the 90-day storage duration, On the other hand, mold counts declined over time, while bacteria levels displayed an initial surge (0 days) and fluctuating decreases throughout the storage period.

Recommendations

Optimizing processing and preservation methods is vital for maintaining food safety, extending shelf-life, and ensuring product quality to protect consumer health. Policymakers should prioritize guidelines for insect-based foods, emphasizing oven-drying and refrigeration as effective interventions.

Author contributions

Christine Mbai did experimental work, analysis of data and manuscript preparation. Professor Hudson Nyambaka, Professor Judith Kimiywe supervised the manuscript's experimental work and revision and approval.

Acknowledgement

We acknowledge the Flemish international council of Belgium for funding the study and making it successful

Disclosure Statement

No conflict of interest was reported by author(s)

Funding

The work was supported by Flemish international council of Belgium

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