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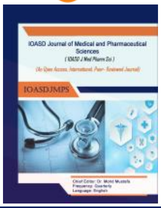

Microbial Analysis of Three-Leaf Yam (*Dioscorea dumetorum*): Microbiological Quality and Spoilage Dynamics During Storage

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ABSTRACT

This study examined the microbiological quality and spoiling development of Dioscorea dumetorum (three-leaf yam), a somewhat obscure yam species produced in equatorial Africa. Cooked yam samples were kept at room temperature and checked for bacterial and fungal development every 0, 24, 48, 72, and 96 hours using conventional microbiological procedures. Over the course of 96 hours, overall viable bacterial counts went from 2.2×10^5 CFU/g to 8.4×10^7 CFU/g, and total fungal counts went from 2.9×10^5 to 2.7×10^7 CFU/g. The most common bacteria were Bacillus cereus and Clostridium perfringens, and the most common fungi were Penicillium spp., Blastomyces spp., and Aspergillus flavus. Sensory test indicated a gradual decline in colour, flavour, and aroma, indicative of microbial growth. The results show that D. dumetorum is quite likely to spoil when stored at room temperature, so it should be eaten right away or kept in the fridge to be safe. The study offers foundational data on the microbial ecology of an underutilised species and elucidates its preservation requirements post-processing.

Keywords: *Dioscorea dumetorum*, microbial load, food safety, spoilage organisms, bacterial count, fungal contamination.

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INTRODUCTION

Yams (Dioscorea spp.) are an important part of West African diets and economies. They are a major source of carbohydrates, important minerals, and dietary fibre for millions of people. They are not only eaten as staple foods, but they are also a culturally important crop that is linked to traditional rites and food security in the area. Nigeria is still the world's top producer of yams, making up more than two-thirds of the entire world production. This shows how important yam farming is to the country's economy and agriculture [1]. Yam is an important part of the Nigerian food system because it is not only an energy food but also helps people in rural areas make a living, trade, and stay healthy.

Dioscorea dumetorum, also known as trifoliate yam, bitter yam, or three-leaf yam, is one of the many yam species grown in tropical Africa. It is unique because its leaves are three-lobed and it has a lot of starch. Even with these traits, D. dumetorum is still one of the least studied and least used species in the Dioscorea genus. People usually eat the tubers after boiling them, and they are known to have a lot of nutrients, such as carbs, proteins, minerals, and bioactive phytochemicals. But the crop can't be used or sold very well because of the problems that come up after harvest, especially the hardening that happens after harvest. This physiological mechanism makes the tubers too hard to eat within a few days of harvest, which greatly lowers their marketability, customer acceptability, and storage life [2].

Microbial contamination and enzymatic activity are often to blame for yams and other root crops going bad after they are harvested. When you leave cooked yam out at room temperature, it is very easy for microbes to get into it because heating eliminates the natural protective layers and moisture barriers. Bacteria, fungus, and other microorganisms use leftover carbohydrates, proteins, and other nutrients as building blocks for their growth. By releasing extracellular enzymes like amylases, cellulases, and proteases, they break down structural polysaccharides and proteins, which makes the product softer, changes its colour, gives it an off-flavor, and eventually spoils it [3]. The practice not only costs money, but it could also be bad for people's health.

There have been reports of pathogenic and toxin-producing bacteria colonising or contaminating cooked starchy meals. Bacillus cereus, Clostridium perfringens, and Aspergillus flavus are prominent examples of organisms associated with foodborne intoxications and infections [4]. Bacillus cereus and Clostridium perfringens are spore-forming bacteria that can survive heat treatment and grow in the right conditions after cooking. Their metabolic processes can generate enterotoxins that cause diarrhoea and vomiting. A. flavus and other Aspergillus species also make aflatoxins, which are very harmful to the liver, cause cancer, and cause birth defects. When these microorganisms are present in cooked yam, especially when it is stored at room temperature, it is a double problem: it spoils and is a public health risk [5].

Even though many people in Nigeria eat a lot of D. dumetorum, there isn't much scientific knowledge about its microbiological quality, how long it stays good after cooking, or how it spoils. Most research have mainly looked at its proximate composition, anti-nutritional factors, and physiological hardening properties. Very few have looked at its microbial ecology or safety concerns. In rural and semi-urban areas without refrigeration, cooked yam is often kept at room temperature for a long time before it is eaten. This behaviour makes it more likely that microbes will grow and toxins will form, especially in tropical areas with high humidity and warmth [6].

To fill this knowledge gap, the current study aimed to assess the microbiological quality and spoiling progression of cooked D. dumetorum tubers during storage at room temperature. The study specifically aimed to identify and quantify the bacterial and fungal populations associated with stored samples, as well as evaluate sensory alterations in texture, odour, appearance, and taste during storage, and examine the implications of the findings for public health and food safety practices. A scientific understanding of the microbial ecology of cooked D. dumetorum will help us make better rules for storing, preserving, and eating this yam species, which is not used very much. This will make food safer, lower the amount of food that goes bad after cooking, and encourage people to use this yam species in a more sustainable way.

MATERIALS AND METHODS

Fresh, mature *D. dumetorum* tubers were harvested from a farm in Ubudom Attanwaebiri, Njaba LGA, Imo State, Nigeria. Tubers were washed, peeled, and boiled until tender. After cooling, the cooked samples were portioned and exposed to ambient room conditions (28 ± 2 °C) for 0, 24, 48, 72 and 96 hours.

Microbiological analyses were carried out immediately after each storage interval. Ten grams of sample were homogenised in 90 mL of sterile distilled water, followed by ten-fold serial dilutions. Aliquots (1 mL) of appropriate dilutions were plated by the spread-plate method on Nutrient Agar (for total bacterial count) and Sabouraud Dextrose Agar (SDA) (for total yeast and mould count). Nutrient-Agar plates were incubated at 37 °C for 24 – 48 hours, and SDA plates at 25 °C for 3 – 5 days. Colony-forming units (CFU) were enumerated and expressed as CFU per gram. Representative colonies were sub-cultured for purification and characterised by standard morphological and microscopic criteria.

Sensory assessment of the stored samples (colour, taste, odour) was performed by a trained panel of five laboratory personnel using descriptive scoring. Statistical evaluation was primarily descriptive owing to the exploratory nature of the study.

RESULTS

Microbial counts

Microbial proliferation increased consistently over the 96-hour storage period (Table 1). The total bacterial count rose from 2.2×10^5 CFU/g at 0 h to 8.4×10^7 CFU/g after 96 h, while total fungal counts increased from 2.9×10^5 to 2.7×10^7 CFU/g. The sharpest rise occurred between 24 and 48 hours, corresponding to visible surface discolouration and the onset of odour.

Table 1: Mean total bacterial and fungal counts of cooked *D. dumetorum* during storage

Storage time (h)	Bacterial count (CFU/g)	Fungal count (CFU/g)
0	$2.2 \times 10^5 \pm 0.4 \times 10^5$	$2.9 \times 10^5 \pm 0.3 \times 10^5$
24	$1.3 \times 10^7 \pm 0.2 \times 10^7$	$3.0 \times 10^5 \pm 0.5 \times 10^5$
48	$6.0 \times 10^7 \pm 0.3 \times 10^7$	$3.3 \times 10^5 \pm 0.4 \times 10^5$
72	$6.8 \times 10^7 \pm 0.4 \times 10^7$	$3.8 \times 10^5 \pm 0.3 \times 10^5$
96	$8.4 \times 10^7 \pm 0.5 \times 10^7$	$2.7 \times 10^7 \pm 0.2 \times 10^7$

Values represent mean ± SD of duplicate determinations.

Predominant microorganisms

Colony morphology and microscopy identified *Bacillus* species as the dominant bacteria throughout storage. *Clostridium perfringens* appeared after 48 h, coinciding with anaerobic odour. Among fungi, *Penicillium spp.* and *Blastomyces spp.* were common at early stages, while *Aspergillus flavus* became prominent after 72 h.

Table 2: Dominant microorganisms isolated from cooked *D. dumetorum*

Storage period	Predominant bacteria	Predominant fungi
0 – 24 h	*Bacillus cereus*	*Penicillium spp.*, *Blastomyces spp.*
48 – 72 h	*B. cereus*, *C. perfringens*	*Blastomyces spp.*, *Aspergillus flavus*
96 h	*B. cereus*, *C. perfringens*	*A. flavus*, *Penicillium spp.*

Sensory characteristics

Observable quality declined in parallel with microbial growth (Table 3). Samples maintained neutral flavour and odour at 0 h but developed sour taste and offensive odour beyond 48 h. Photometric colour index declined from 0.31 to 1.67 after 96 h, indicating browning and deterioration.

Table 3. Sensory and colour changes of *D. dumetorum* during storage

Storage time (h)	Colour index (mean)	Taste description	Odour description
0	0.31	Neutral / Earthy	Pleasant vegetal
24	3.03	Slightly unpleasant	Earthy neutral
48	2.40	Slightly sour	Unpleasant
72	2.12	Sour	Offensive
96	1.67	Strongly sour	Putrid

DISCUSSION

The investigation showed that the number of microbes in cooked D. dumetorum held at room temperature went up a lot over time. This fast growth is due to good environmental circumstances, such as moderate humidity, adequate nutrients, and no refrigeration. All of these things help bacteria and fungi proliferate.

The prevalence of Bacillus cereus aligns with previous studies regarding the microbiology of cooked starchy foods in West Africa [7]. B. cereus spores can survive boiling and grow while being stored, which makes toxins and can cause food poisoning (8). Clostridium perfringens, an anaerobic spore-former, also does well in micro-environments with low oxygen levels that are formed by dense yam tissues. The presence of these organisms beyond 48 hours strongly suggests that prolonged storage under ambient conditions may render D. dumetorum dangerous for ingestion [8].

Fungal isolates, including Penicillium and Blastomyces, emerged early but predominantly functioned as surface pollutants. However, the subsequent appearance of Aspergillus flavus is worrying from a toxicological point of view. A. flavus makes aflatoxins, which are strong carcinogens that can cause liver cancer and are often found in grains and tubers that aren't stored properly [9]. Finding this species after 72 hours shows how important it is to follow stringent hygiene rules and store things properly.

After only 24 hours of storage, the microbial counts were higher than the maximum allowed values for ready-to-eat meals ($\leq 10^5$ CFU/g) defined by the International Commission on Microbiological Specifications for meals (ICMSF) [10]. This shows that D. dumetorum goes bad quickly once it is cooked. The concurrent alterations in sensory attributes—sour flavour and unpleasant odor—are associated with microbial metabolism that leads to the generation of acids and volatile compounds [11]. Darkening of colour may occur due to oxidative enzymatic processes in conjunction with microbial pigment synthesis.

Earlier research on alternative yam species, like D. rotundata and D. alata, have demonstrated similar patterns, although the rotting process was slower because these species had less moisture and a different starch content [12]. D. dumetorum is more susceptible to disease, which may be because it is softer and can hold more water, making it a great place for microbes to flourish.

Preventive measures encompass immediate eating within 12–24 hours post-cooking, moisture reduction through drying, or storage at refrigerated temperatures (< 4 °C). Natural compounds like ginger and clove have also been demonstrated to slow down the growth of microbes in yam products [13]. Additional research could examine the effectiveness of these bio-preservatives in D. dumetorum.

The study's shortcomings encompass the lack of molecular identification methodologies and quantitative toxin testing. Nonetheless, the morphological methodology utilised yields essential insights into the rotting microbiota and supplies significant baseline data for forthcoming molecular or metagenomic studies.

CONCLUSION

When stored at room temperature, cooked Dioscorea dumetorum shows rapid growth of microbes and loss of taste. The main organisms—Bacillus cereus, Clostridium perfringens, Penicillium spp., Blastomyces spp., and Aspergillus flavus—show that food can rot and be unsafe to eat. After 24 hours of storage, the number of microbes is too high for safe eating.

To reduce the risk of contamination, it is best to eat food right after cooking, keep proper hygiene when handling it, and store it in the fridge. The results show how important it is to keep an eye on microbes in root crops that aren't used very often, and they support attempts to make food safer after harvest in tropical areas.

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