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Enumeration and Identification of Fungi from Selected Local Spices Sold in Minna Nigeria

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Abstract**:** Spices are defined as any plant substance either in powdered form, broken or as a whole which have aromatic features capable of influencing a change of taste or smell of food and beverages. This study was aimed at enumeration and identification of fungi from selected food spices from Minna Nigeria markets. Samples of ginger, chilli pepper, turmeric, nutmeg, locust beans, black pepper, clove and garlic were collected randomly from two different markets; Kure and Bosso in Minna metropolis. The proximate analysis of the samples (moisture, total ash, crude fiber, fat, and carbohydrate) was determined. Standard microbiological techniques were used to enumerate, isolate and identify the fungi in the samples. The proximate composition showed that the highest moisture content was found in turmeric (15.85 %). The highest ash content was in cloves (9.70 %). The highest crude fibre was present in chilli pepper (10.69 %) and the highest carbohydrate was found in garlic (74.07 %). Locust beans had the highest oil extract (24.60 %) and crude protein (22.75 %). The highest fungal count was obtained in nutmeg (1.36×104 cfu/g) followed by ginger (9.2x103 cfu/g). The lowest fungal count was obtained in turmeric and garlic (1x102 cfu/g). The fungi isolated and identified were as follows: *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *A. terrus*, *Saccharomyces cerevisiae* and species of *Aspergillus*, *Mucor*, *Penicillium*, *Trichophyton*, and *Chrysosporium*. The results of this study showed

that the selected local spices harbour different fungal species. Therefore, proper handling hygiene and storage is recommended.
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Keywords: local spices, proximate composition, fungal count, fungal species, and Minna

1.0 Introduction

Often times, one may be wondering what makes most foods have peculiar taste, aroma, flavor and even color. It is simply because of the presence of what is known as food additives, among which spices are of great significance in influencing how food products taste and smell. Spices have become an important component of the human food; they are found in diverse cultures of the world [1].

Spices are fresh, ripe, dried, or granulated farm produce that make up vital group of agricultural goods, which are essential in cookery, as they are been employed as flavor, taste and color enhancers. Spices may exist as seeds, fruits, flowers shoots, stigmas, buds, barks, leaves or as roots. Over the years, spices have been employed as reserves for pharmaceutical preparations and as dietary supplements and antioxidant in various industries such as pharmaceutical and food. Spices are defined by El-Gali [2], Nguegwouo et al. [3] and Osman et al.[4] as any plant substance either in powdered form, broken or as a whole, which have aromatic features capable of influencing a change of taste or smell of food and beverages. Spices as described by Kabak and Dobson [5] are the parts of plants except the leaves, which are utilized as seasonings, flavoring and coloring agents of food. Spices can be any part of plant such as bark from cinnamon, buds from cloves, berries as grains of pepper, root from ginger, seeds such as cumin and stigma of flowers such as saffron [5]. Spices have the ability to entice someone who is unwilling to eat or drink any food product to want to take more after tasting the food [6]. Some commonly used spices in Nigeria include, black pepper (Piper nigrum Linn.) [7], cloves (Syzygium aromaticum, or. Eugenia aromaticum or Eugenia caryophyllata) [8], Ginger (Zingiber officinale) [9], turmeric [10], chilli pepper [11], garlic (Allium sativum L.) [7], and locust beans (Parkia biglobosa) [12].

Even before the medieval era, spices have been used and they have significant function in antiquities civilization. Most spices have been traced to have originated from the tropics of Asia where they have been used to influence aroma, flavor, color and also play part in preservation of food and beverages [1]. In Nigeria, one of the most widely used species of plants are the spices since they are widely used in every home during preparation of meals, which may come in the form of seeds and/or powders with the ability to influence the taste and smell of foods and drinks preparation [1]. Aside food, spices play important function in folk medicine, cosmetics, perfumes and used as customary rites such as marriages [13].

Among the Hausas in Nigeria, spices have been used in the bathing of newborn babies as well as in the preparation of food for pregnant women and women who newly delivered to help boost their immune system [13]. Spices are also used as antimicrobial agents such as ginger and garlic. One may still be wondering, why these spices still get spoiled over some

time despite having antimicrobial properties. Microbial spoilage of these spices often occurs as a result of unhygienic practices, poor processing and poor storage [1].

With events of food poisoning occurring across the globe, food security has been of paramount importance in order to avoid outbreak such as that which happened in Kenya in 2004 where 125 persons were reported to have died from consumption of maize contaminated with mycotoxin known as aflatoxin [14]. The presence of pathogenic microorganisms and/or their metabolites in food is often detrimental to the health of humans as some of them such as Salmonella, Pseudomonas, Escherichia coli, Shigella among others, have the ability to cause havoc to people who ingest food contaminated by these microorganisms [15].

Most spices are preserved in their dehydrated form, yet microorganisms such as fungi that in one way or the other find their way to these spices can remain inactive by forming dormant spores, which may become active in contact with moisture. Fungi such as the genus Aspergillus have this ability to cause spoilage even at low water activity in most spices provided there is little contact of the spices with water [16]. It becomes more difficult to control contamination once low-moisture food or the food environment is contaminated. Some of the reasons for this include: enhanced resistance of a number of microorganisms to drought rather than wet heat, challenges of pathogen detection in final product testing (e.g., pathogens are not homogeneously distributed and during enumeration pathogens may be outcompeted by non-pathogenic species), longer survival time of microorganisms in low-moisture food, possibility of spore germination and pathogen growth during rehydration, as well as public perception that dry foods are sterile, which may lead to careless handling practices. The climate where spices are cultivated and the conditions under which they are cultivated, harvested, processed and stored contribute to the possibility of contamination of spices [1].

Despite effort put in to prevent food poisoning, fungal genera such as Penicillium, Fusarium, and Aspergillus still pose serious health risk due to their ability to produce mycotoxins particularly aflatoxins which triggers toxic immune response capable to cause damage of tissues, organs and possibly death to humans as well as animals [17]. The aim of this study was to enumerate and identify fungi species from selected food spices sold in Minna Markets.

2.0 Materials and Methods

Study area

The study was conducted in the Department of Microbiology, Federal University of Technology, Minna, Nigeria. Minna is a large community in North Central Nigeria, with the population exceeding 300,000 people. It has two large ethnic communities, the Gwaris and Nupes and located on geographical coordinates of 9°36'54" North, 6°32'51" East.

Collection of samples

Samples of the following spices: ginger, chilli pepper, turmeric, nutmeg, locust beans, black pepper, clove and garlic, were collected randomly from two different markets; Kure and Bosso in Minna metropolis. Spices sold in these market places are usually kept in wooden boxes, gunny bags, containers made of plastic or metals, polythene bags, and

sometimes on the bare ground. During sample collection, care was taken to avoid collecting samples showing any visible sign of deterioration. The samples were bought in grounded form and were kept in a polythene bag. The samples were brought into Microbiology Laboratory, Federal University of Technology, Minna for analysis.

Proximate composition analysis

The proximate analysis of the samples for moisture, total ash, crude fiber and fat was carried out in triplicate using methods described by Onwuka [18]. The nitrogen was determined by micro Kjeldah method described by Onwuka [18] and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrate content was estimated by calculating the percent remaining after all the other components have been measured. All the proximate values were reported in percentage (%).

Enumeration of fungi

Standard dilution plate method was used to isolate and analyze fungi from the spices as described by Toma and Abdulla [19]. One gram of each composite sample (fine powder) was transferred into 20 mL of sterile sample container containing 9 mL of sterile distilled water and was mechanically shaken for 15 min in order to homogenize the sample. The suspension was allowed to stand for 10 minutes, then a serial dilution was carried out by transferring 1 mL from the first test tube into second test tube containing 9 mL of distilled water. The second was mixed gently, and 1 mL was taken from the second test tube into the third test tube and to the fourth test tube.

The organism was isolated using pour plate method. One millilitre (1 mL) of each diluent from the second test tube and the fourth test tubes was pipetted using a new sterile pipette into Petri dishes. After samples have been poured into plates, 15 mL of sterile Sabouraud dextrose agar (SDA) was poured into the plates and swirled. The media was allowed to cool to 45°C before adding 250 mg of antibiotic (chloramphenicol) for 250 mL of SDA to prevent bacterial growth. The SDA plates were incubated at 28°C±2 for 3-5 days and monitored every 24 h. The isolates were counted by visual observation and multiplied by inverse of the dilution factor. The result was expressed as colony forming unit (cfu) /g of samples. All plates were examined macroscopically and microscopically. Representative colony types were purified by sub culturing on fresh SDA plates. Pure cultures were transferred to a slant bottle of SDA. Pure cultures of the isolates were grown singly on SDA for identification.

Identification of the fungal genera

Identification of fungal genera was carried out as explained by Toma and Abdulla [19]. The fungal isolates were transferred to sterilized plates for purification and identification. The fungal isolates were examined macroscopically by colonial morphology (e.g colour, fluffiness, reverse dise colouration, dry or moist) and under microscope for spore arrangement, hypha structure, sporangiophore shape and size. The morphological characteristics and appearance of the fungi were identified in accordance with methods employed by Adebayo-Tayo et al. [20].

3.0 Results

The proximate component of local spices is shown in Table 1. The proximate composition of local spices shows that the spices are of high nutritional values. The highest moisture content (%) was

obtained in turmeric (15.85±0.75). The highest ash content (%) was obtained in clove (9.70±0.25). The highest crude fiber was obtained in chili pepper (10.69±0.45). The highest crude protein (22.75±0.90) and the oil extract (24.60±3.30) were obtained in locust beans. The values with different superscript differ significant ($p<0.05$) while value with the same superscript do not differ significantly ($p>0.05$) from each other. The log of viable fungal counts in the local spices is shown in Figure 1. The highest count was obtained in nutmeg (1.36×104 cfu/g) (Log count of 4.13 cfu/g), then followed by ginger 9.2x103 cfu/g (Log count of 3.96 cfu/g). The lowest colony count was obtained in turmeric and garlic (1x102 cfu/g) (Log count of 2.0 cfu/g).

Table 1: Proximate composition analysis of local spices

Sample	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	Oil Extract (%)	Carbohydrate (%)
Ginger	9.85±0.25^{ab}	8.83±0.20^{c}	8.75±0.30^{d}	5.30±0.15^{c}	4.35±0.10^{a}	62.88±5.50^{e}
Chilli Pepper	8.65±0.35^{a}	6.65±0.25^{b}	9.62±0.40^{e}	10.69±0.45^{e}	16.54±0.50^{c}	47.83±1.00^{b}
Turmeric	15.85±0.75^{d}	6.85±0.20^{b}	7.00±0.18^{c}	4.30±0.15^{b}	12.13±3.00^{b}	53.88±0.60^{cd}
Nutmeg	10.85±0.28^{b}	9.35±0.32^{d}	8.75±0.25^{d}	6.28±0.29^{d}	11.90±0.35^{b}	52.69±4.57^{c}
Locust beans	12.25±1.82^{c}	3.80±0.06^{a}	22.75±0.93^{g}	5.65±0.15^{c}	24.60±3.30^{d}	31.85±2.00^{a}
Black Pepper	14.28±0.22^{d}	3.81±0.13^{a}	13.12±0.55^{f}	5.28±0.31^{c}	5.60±0.15^{a}	57.91±0.63^{d}
Clove	8.70±0.70^{a}	9.70±0.25^{d}	5.20±0.44^{b}	1.35±0.10^{a}	16.80±0.89^{c}	58.25±0.28^{d}
Garlic	10.57±0.51^{b}	6.59±0.24^{b}	3.93±0.20^{a}	1.61±0.90^{a}	3.25±0.63^{a}	74.07±0.14^{f}

Values with different alphabets in the same column are significantly different ($P < 0.05$) from each other, but values with same superscripts do not differ significantly from each other ($P > 0.05$)

The morphological characteristics of fungi isolates are shown in Table 2. Several characteristics (colour and arrangement of spores, aerial hyphae, growth, fluffiness, reverse dise colouration, dry or moist), hypha structure, sporangiophore shape and size) were observed and recorded. The probable identity of microbial isolates is listed after examining several characteristics in Table 2. The result shows that identified fungal genera associated with local spices include, *Aspergillus niger, A. fumigatus, A. flavus, A. terrus, Saccharomyces cerevisiae* and species of *Aspergillus Mucor, Penicillium* and *Trichophyton*

.

Table 2 Fungi isolated from the local spices in Minna

Isolate code	Sample name	Suspected organism
A	Ginger	*Mucor* sp.
B	Chilli pepper	*Aspergillus terrus, Chrysosporium* sp. *Aspergillus* sp., *Trichophyton* sp.
C	Turmeric	*Aspergillus fumigatus, Aspergillus flavus*
D	Nutmeg	*Saccharomyces cerevisiae*
E	Locust beans	*Aspergillus flavus, Trichophyton* sp.
F	Black pepper	*Aspergillus niger, Aspergillus* sp.
G	Cloves	*Penicillium* sp., *Aspergillus flavus*
H	Garlic	*Mucor* sp.

4.0 Discussion of Findings

Spices have been reported to possess antimicrobial properties [21-23], however, microorganisms have evolved mechanism to evade these properties making them able to contaminate various food materials including spices. The proximate content of the spices in the study showed that they are of high nutritional value supplying various nutrients to the consumers. Aside that, they also supply nutritional requirements for the growth of microorganisms including fungi. For this reason, the proximate result obtained in this study supports the findings as reported by Lippolis et al. [16] where different fungi genera were found present in the various spices analyzed.

The proximate composition of spices showed that the highest moisture content was obtained in turmeric, which is in contrast to the reports of Tchiegang and Mbougueng [24] where in their findings, chilli pepper had the highest moisture content. This may be due to the capacity of the tissues of turmeric to retain higher amount of water in the initial phase before they were dried compared to the chilli pepper. Another factor to be considered is the period/duration used in the drying, as short time drying period may result in high moisture content though the method of drying used goes a long way to determine the amount of moisture that could be left after the period of exposure to heat.

Moisture content is an important factor that determines the extent of microbial contamination of spices since it measures the stability and susceptibility of the spices to microbial attacks [16]. Therefore, the low moisture content remains an asset in storage and preservation of nutrients because higher moisture content could lead to food spoilage through increasing microbial action [1].

Another requirement for microbial growth is the presence and level of minerals available in their environment. This in spices was ascertained by determining the ash content (%), which was reported to be highest in clove in this study. This result supports the findings of Effiong et al. [25] where cloves appeared to have the highest ash content indicating the presence of nutritionally important minerals [26].

Information concerning the fiber content of chilli pepper and how they contribute in aiding digestion process have become a point of interest to nutritionist [27] as this study showed the presence of high fiber content in chilli pepper than other spices. This result differs from the one obtained in the study carried out by Ameh et al. [28], Ogunlade et al. [29], Sharma et al. [30] and Esayas et al. [31]. This could be due to differences in season, time of harvest, variety or geographical location.

The highest carbohydrate was obtained in garlic. Petropoulos et al. [32] reported that dry garlic bulbs mainly consist of carbohydratesThe lowest crude protein and oil extract was obtained in garlic and ginger respectively, which is similar to the reports of Tchiegang and Mbougueng [24] and Petropoulos et al. [32]. A total of six fungi were isolated using the agar plate method. These were Saccharomyces cerevisiae and species of Mucor, Penicillium, Chrysosporium, Trichophyton and Aspergillus. Aspergillus sp. was commonly isolated in most of the spices. This may be due to the ability of Aspergillus sp. to withstand adverse conditions perhaps due to the presence of resistant spores

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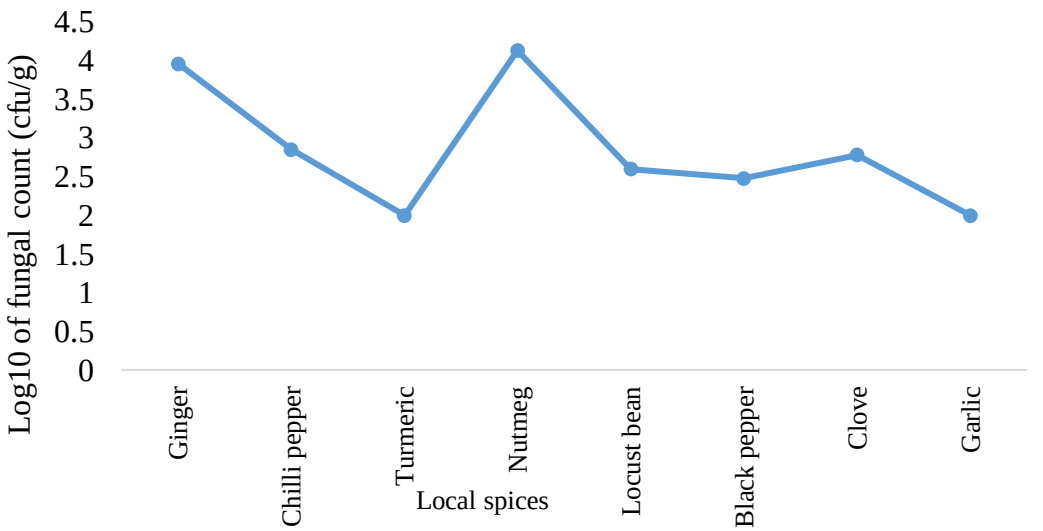

Figure 1. Log10 of fungal counts in the local spices

This result is similar to the one reported by Toma and Abdulla [19]. The highest fungal count was obtained in nutmeg followed by ginger which is in contrast to the reports of El-Gali [2]. This may be due to the differences in nutritional composition, methods employed in the processing, as well as the storage conditions to which the spices were exposed. Though reports from Duarte et al. [33] showed that nutmeg had high antifungal activities, however, what was obtained in this study was different. This may be attributed to the conditions in which thesamples were analyzed. In this study, dried form of nutmeg was used, where most of the essential oil reported to confer the antifungal activities in other

study such as that of Duarte et al. [33] may not be present to inhibit fungal growth. The lowest colony count was obtained in turmeric and garlic, which is in agreement with other studies such as the one reported by Toma and Abdulla [19] and Haruna et al. [34]. The bioactive compound in turmeric (curcumin also known as diferuloylmethane) and garlic (allicin and organosulfur) have been reported by D'Souza et al. [35] and Singh and Singh [36] to have strong antimicrobial properties, which may be the reason for the low fungal contamination of the spices. Locust beans showed the highest proximate result for crude protein and the oil extract. Locust beans have been reported to have high nutritional and health benefits, which is evidenced by the proximate result. As such was expected to support high amount of fungal growth. This was also reported by Nwadiaro et al. [12]. However, low fungal count was observed regardless of the high nutrient content in the locust beans. This goes a long way to applaud the method of processing as well as the level of hygiene employed by the spices sellers. Another factor, seemingly contributing to the low fungal contamination may be the presence of lactic acid probiotic producers, which could induce acidic environment and in which some fungi may not survive [37-38].

5.0 Conclusion

The result of the study shows that the selected local spices has a high nutritional content and harbour different fungi isolates.that the selected local spices has a high nutritional content and harbour different fungi isolates harbour different fungi isolates. that the selected local spices has a high nutritional content and harbour different fungi isolates. The fungi isolated and identified are as follows Aspergillus niger, A. fumigatus, A. flavus, A. terrus, Saccharomyces cerevisiae and species of Aspergillus, Mucor sp., Penicillium, Trichophyton and Chrysosporium. Aspergillus sp. were identified in most of the spices. Considering the implications of the observed counts and presence of fungi in the spices, proper handling hygiene and storage is recommended. This may preclude the presence of pathogenic fungi and reduce microbial counts to the lowest numbers.

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