JOURNAL OF MULTIDIMENSIONAL RESEARCH & REVIEW

Website: http://www.jmrr.org **Sep (2019) Vol.1, Iss.2 Pages:91-104**

EFFECT OF DIFFERENT STORAGE CONDITIONS ON THE MICROBIAL PROFILE OF SOME COMMONLY USED SPICES IN NIGERIA

Omorodion Nnenna JP

University Of Port Harcourt P.M.B 5325 Port Harcourt Rivers State Nigeria

Article Received: July 2020 Published: September 2020

Abstract

Spices is an important condiment in food preparation as increases the aroma and palatability of food. Medicinal and antimicrobial properties of spices have been widely researched. However, the microbial profile and most appropriate method of spices preservation and storage are rarely known. In this study 5 spices samples namely Nutmeg, Uda, (Ngero pepper) Uziza seed (Ashanti pepper),Ginger, Garlic were analysed microbiologically after 7 day of storage at different condition (refrigerator, exposed, covered and stored at room temperature and sundried. For total heterotrophic count, coliform count, Staphylococuss count and fungi count. The ranged from 1.0x10cfu/g for sundried uda to 1.68x107 cfu/g for refrigerated nutmeg. A total of 104 bacteria isolates which include Bacillus40(3.48), Micrococcus25(24.0%) Staphylococcus 24(23.07%), Citrobcater4(3.84%), Ecoli2(1.9%), Serretia(4,(3.8%), Proteus3(2.8%) were recovered .Staphylococcus count was sundried samples, while fungi count ranged from 1.9x104 cfu/g for fresh uziza to 6.2x104cfu/ml for covered ginger stored at room temperature from which a total of 77 fungi isolates comprising of Aspergillus, Sacchromyces, Rhizopus, Penicillum and Fusarium were recovered from all spices samples Because of the widespread use of spices There is a need to stress the importance of correct handling of food which incorporate spices both at a domestic and commercial level. A Need is evident to establish standards of compliance for Spices to give the user a reliable safe product.

Keywords: Spices, anti microbial, microbiology

1. INTRODUCTION

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste (Geneva, 1982).Spices are often used in perfumes and cosmetics and many have been used in medicine and religious rituals as well. Spices are distinguished from other plant products used for similar purposes, such as herbs which are green leafy parts of plants, aromatic vegetables and dried fruits (Wright, 2002). However as microbiologist one is hardly interested in the extra flavour or aroma species could add to food; rather as a microbiologist one is interested in: Antimicrobial properties of the spices. Many researchers have worked on the ability of spices to inhibit the growth of microorganisms, also in so many works the particular substance that convey this antimicrobial property and their mechanism of action have been studied.Microbial composition of the species which could either be the normal flora of the species or contamination due to harvest or post harvest practices or poor sanitary condition.Sometimes as microbiologist one could be interested in the nutritional benefits of the spices .As with many other agricultural products, herbs and spices may be exposed to a wide range of microbial contaminants before, during and post-harvest (McKee, 1995; Koci-Tanackov et al., 2007). Although used in small quantities, herbs and spices are recognized as significant carriers of microbial contamination primarily xerophilic storage mold and some bacteria (Dimic et al.,2000, Romagnoli et al., 2007).

Fungi are the most common contaminants, most are probably commensal residents of the plant. Spices are collected in tropical areas using a traditional method, which means that the products are exposed to contaminants from the soil and air, before being sufficiently dry to prevent microbial growth (Kneifel and Berger, 1994), as well as during harvesting, handling and packing.A spice may be available in several forms; fresh, whole dried, or pre-ground dried. Generally, spices are dried. The type of spice and their storage conditions determine their shelf life. Whole spices have longer shelf life than ground spices.

Spices are stored in airtight containers to protect against moisture and preserve oils that gives spices rich flavour and aroma. Spices are also stored in a cool, dry place, away from exposure to bright light, heat or moisture. But of all reason, those mentioned above inclusive, the reason and objective of this research work is to determine the microbial composition of (five) different species under various methods of storage and preservation technique, which include refrigeration, sun drying and storing at room temperature. The microbial composition of freshly bought species was compared to that of the store under the above mentioned conditions for seven days. The species used in this research work include: uda, garlic, ginger, uziza Determination of the total fungal and total heterotrophic bacterial population of the spices, total Staphylococcus and coliform count population of the spices,To compare the level of microbial load of each spices based on the effect of their storage condition (exposed, refrigerated and stored in a container).

2. MATERIALS AND METHOD

SAMPLE COLLECTION

Five spice samples; ginger, garlic, black peppercorn (uziza seed), nutmeg (*Myristica fragrans*) and *Xylopia aethiopica* (uda) were bought and transported to the Microbiology laboratory in the University of Port Harcourt for microbiological analysis.

SAMPLE PREPARATION

10g of all samples freshly bought from the market was used to prepare a stock solution, after which serial dilution was carried out. A fraction of all five spice samples: ginger-GG, uda-UD, uziza seed-UZ, garlic-GL and nutmeg-NM was stored in the refrigerator with a sub letter code 'r' to represent refrigerator. Another fraction all spice sample was stored with a covering at room temperature with a sub letter code 't' to represent room temperature. Another fraction was sundried and sub coded 's'. Another fraction was stored exposed at room temperature. All sample under various preservation methods were stored for 7 days.10 g of each spices sample were added into 90 ml of peptone water, swirled and allowed to stay for few minutes after which a ten-fold serial dilution was done by pipetting 1 ml from the stock solution into the next test tube (10^{-2}), the process was done repeatedly up to (10^{-5}).

ISOLATION OF MICROORGANISMS

10grams of each species samples stored at the various storage conditions (sundrying, refrigeration, storing at room temperature in a covered container, and storage at room temperature exposed) and the fresh samples was homogenized in 90mls of peptone water for preparation of stock culture to be used for serial dilution.

Total bacterial count

0.1 aliquot of 10^{-5} and 10^{-6} dilution of the various spice sample at different storage conditions was cultured into Plate count agar in duplicates and incubated at 37C for 24-48hrs

Coliform Count

0.1 aliquot of 10^{-3} and 10^{-4} dilution of the various spice sample at different storage conditions was cultured into MacConkey agar in duplicates and incubated at 37C for 24-48 hours.

Staphylococcal count

0.1 aliquot of 10^{-3} and 10^{-4} dilution of the various spice sample at different storage conditions was added into Manitol salt agar (MSA) in duplicates and incubated at 37C for 24-48 hours.

Single colonies of bacteria growth were randomly selected from different media plates based on their morphology and were subcultured and incubated at 37^{o}C for 24 h to obtain pure colonies.

IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL ISOLATES

Isolates were identified based on their morphological and cultural characteristics on growth media. Identification materials, reagents and protocols according to (Cheesebrough, 2000) were used to identify discrete colonies from the bacteriological media of sub-cultured isolates

Fungal count

aliquot of 10^{-3} and 10^{-4} dilution of the various spice sample at different storage conditions was added into potato dextrose agar (PDA) in duplicates and incubated at at 27C for 3 da

IDENTIFICATION AND CHARACTERIZATION OF FUNGI ISOLATES

The cultural characteristics of each fungi isolates were identified according to their colour, shape and the cell morphology was done based on mycelia, hyphae, septate, spore formation using lactophenol blue. A piece of the mycelium from the Petri plates was mounted on a clean grease free slide using a sterile wire loop and covered with a cover slip, after which a drop of lactophenol cotton blue was added and examined with the microscope.

3. RESULTS

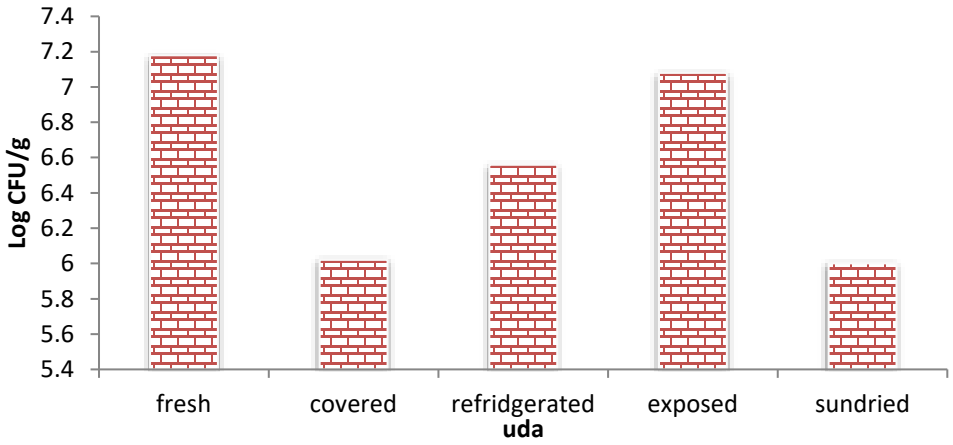

Fig 1 Bar chart of the total viable count of uda spice at different storage condition

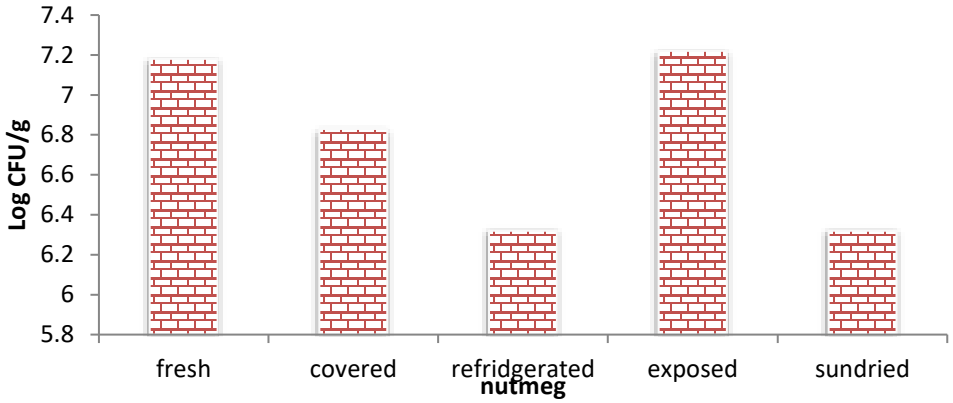

Fig 2 Bar chart of the total viable count of nutmeg spice at different storage conditions

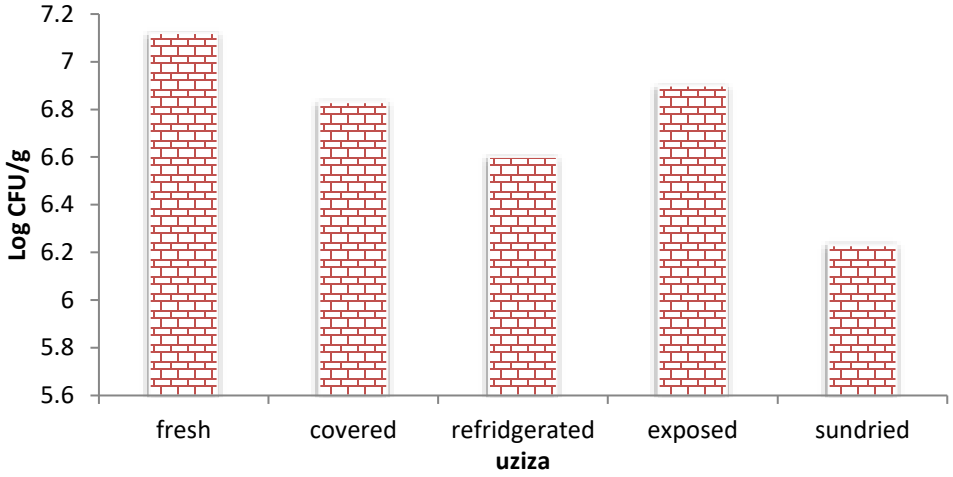

Fig 3 Bar chart of the total viable count of uziza spice at different storage conditions

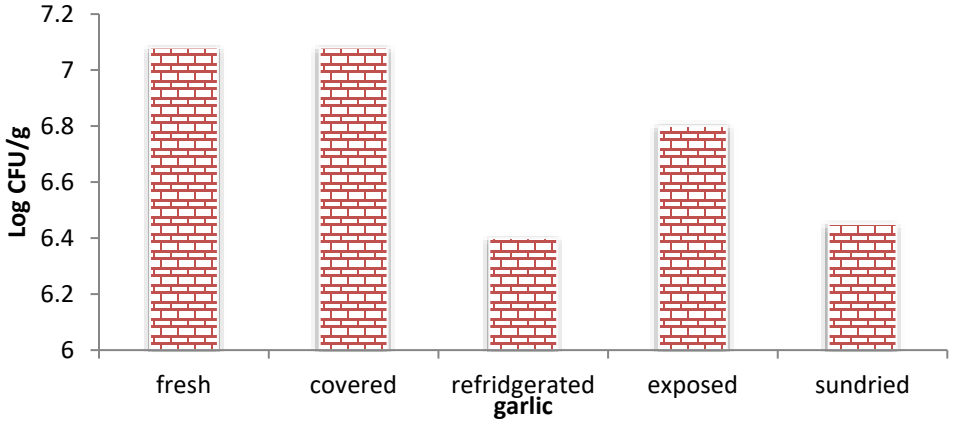

Fig 4 Bar chart of the total viable count of garlic spice at different storage conditions

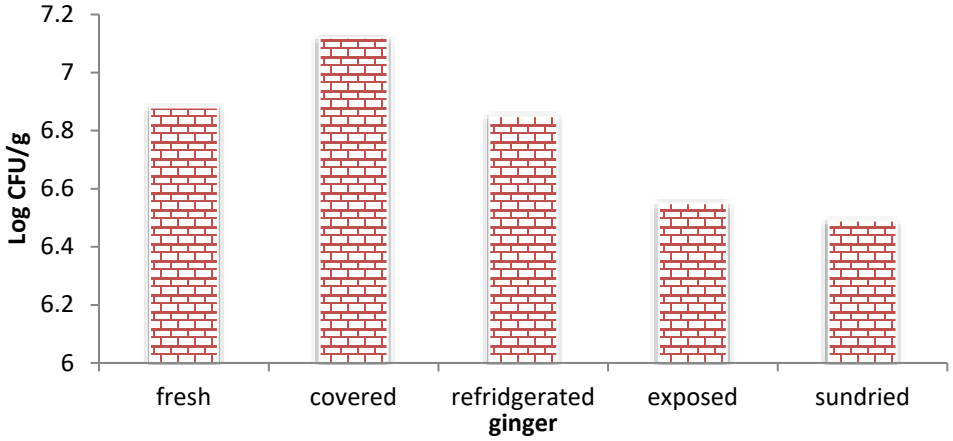

Fig 5 Bar chart of the total viable count of ginger spice at different storage conditions

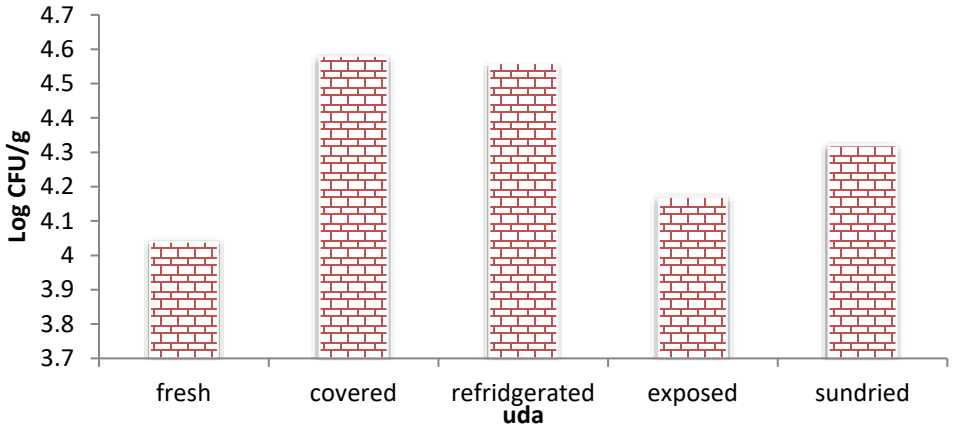

Fig 6 Bar chart of the total fungal count of uda spice at different storage conditions

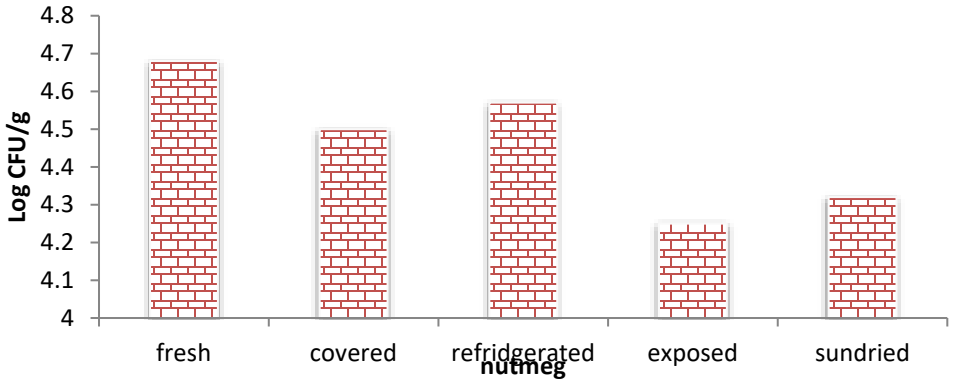

Fig 7 Bar chart of the total fungal count of nutmeg spice at different storage conditions

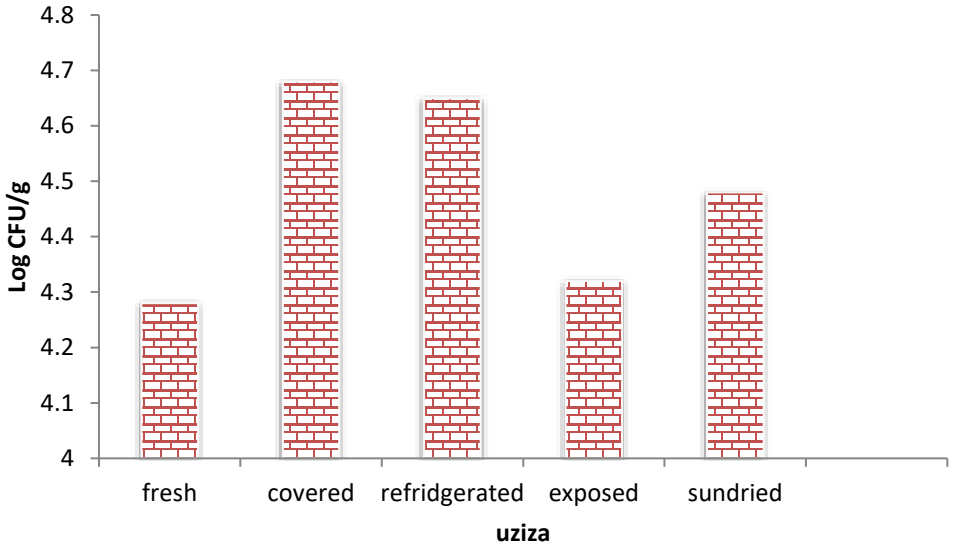

Fig 8 Bar chart of the total fungal count of uziza spice at different storage conditions

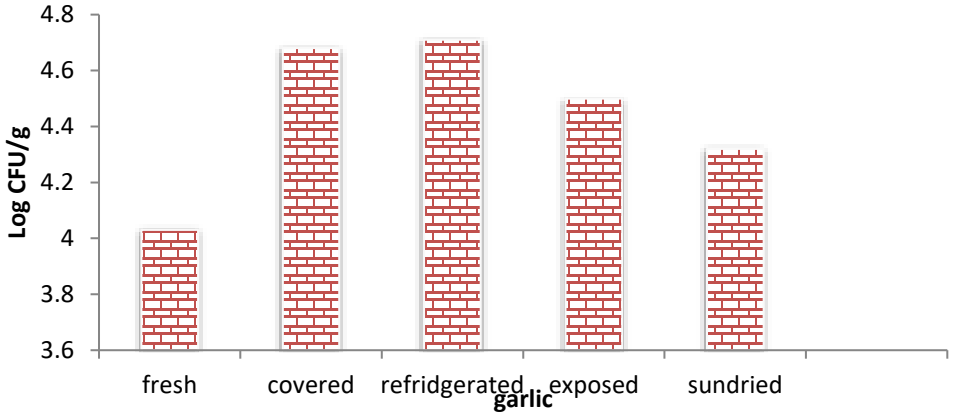

Fig 9 Bar chart of the total fungal count of garlic spice at different storage conditions

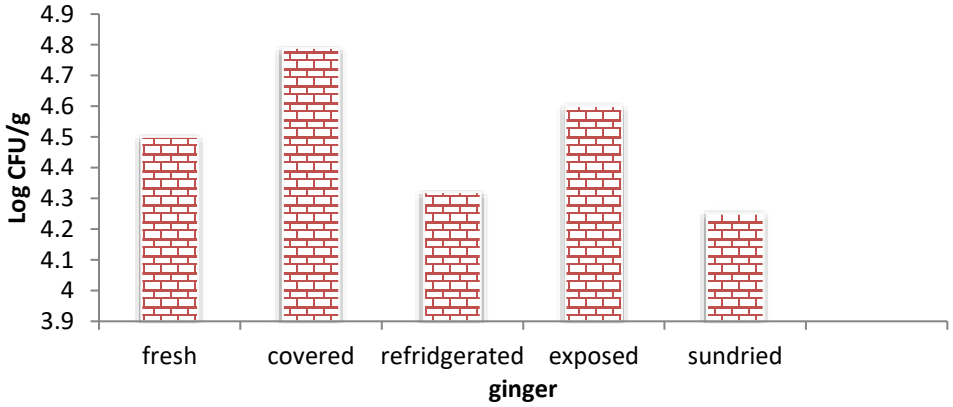

Fig 10 Bar chart of the total fungal count of ginger spice at different storage condition

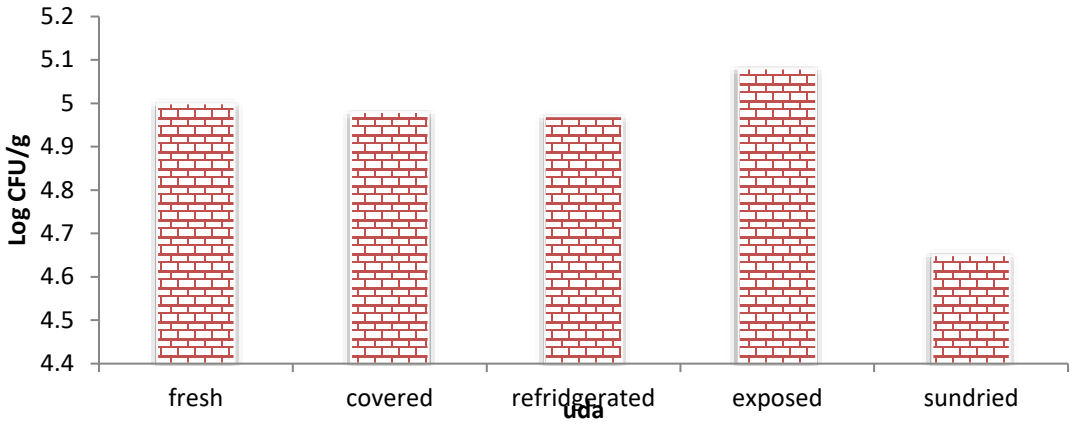

Fig 11 Bar chart of the total Staphylococcus count of uda spice at different storage conditions

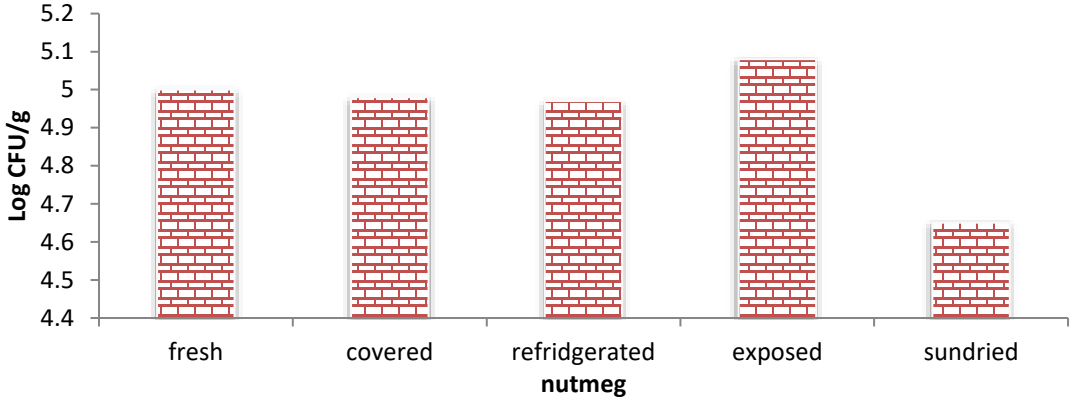

Fig 12 Bar chart of the total Staphylococcus count of nutmeg spice at different storage conditions

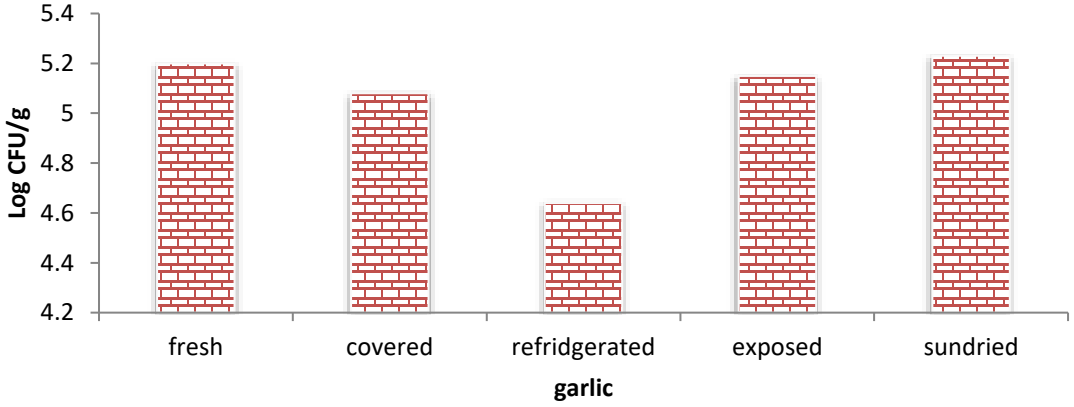

Fig 13 Bar chart of the total Staphylococcus count of garlic spice at different storage condition

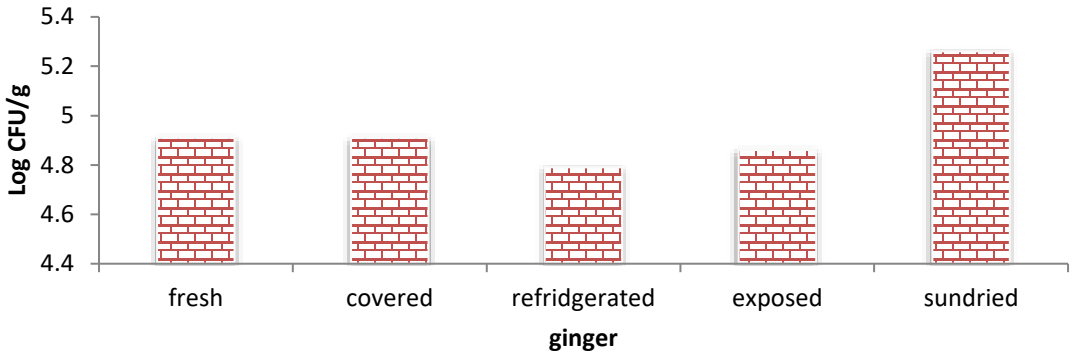

Fig 14 Bar chart of the total Staphylococcus count of ginger spice at different storage conditions

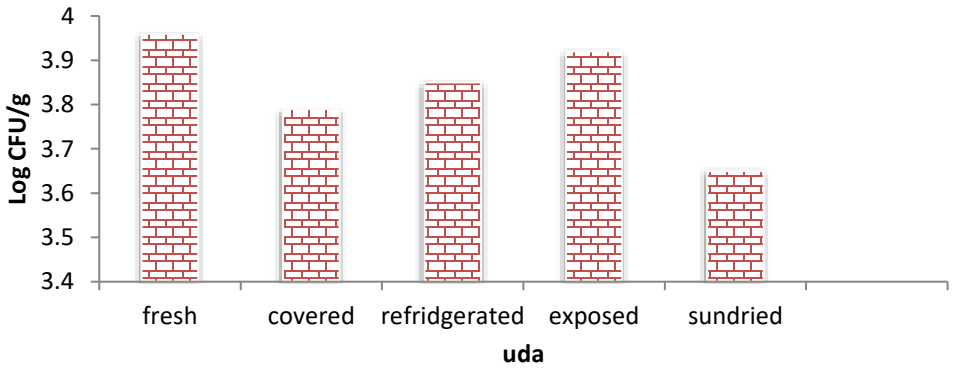

Fig 15 Bar chart of the total coliform count of ginger spice at different storage conditions

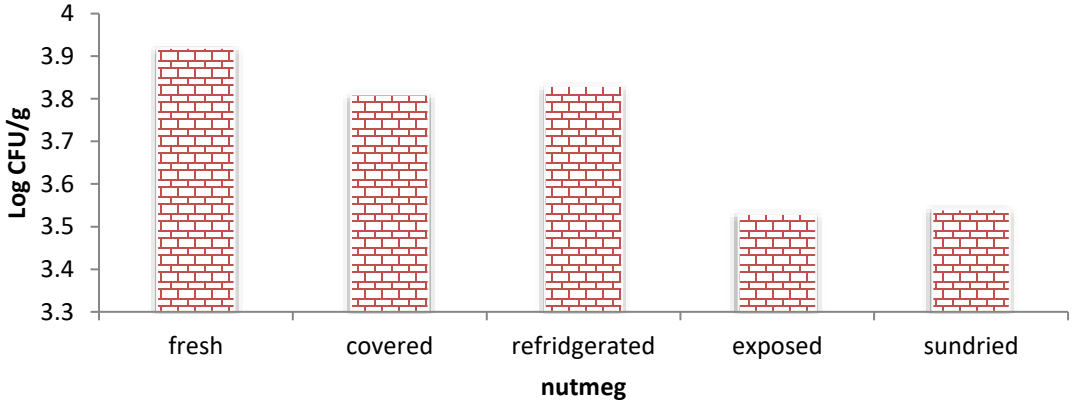

Fig 16 Bar chart of the total coliform count of nutmeg spice at different storage conditions

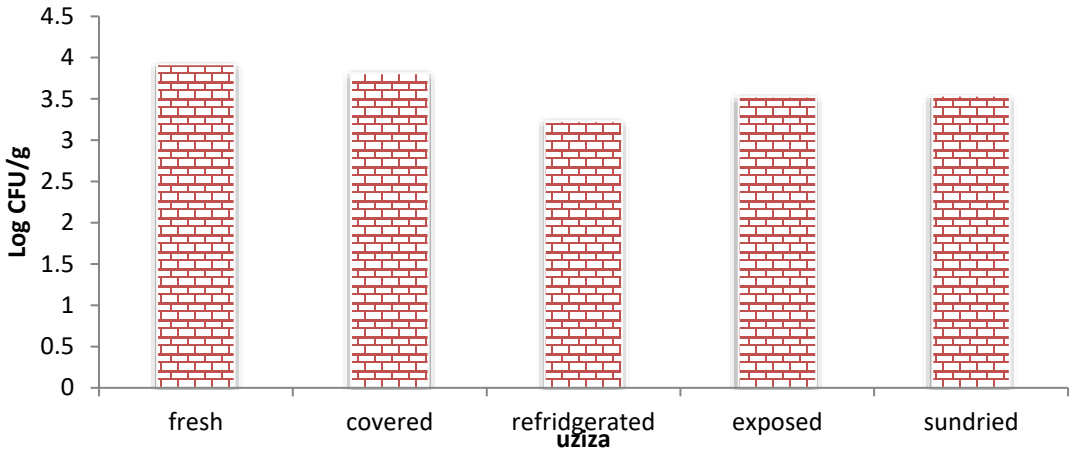

Fig 17 Bar chart of the total coliform count of uziza spice at different storage conditions

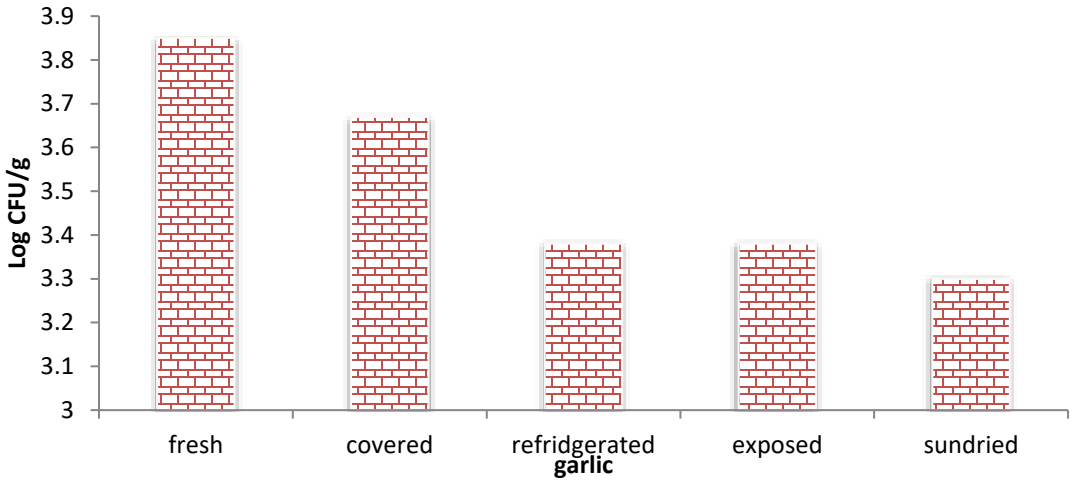

Fig 18 Bar chart of the total coliform count of garlic spice at different storage conditions

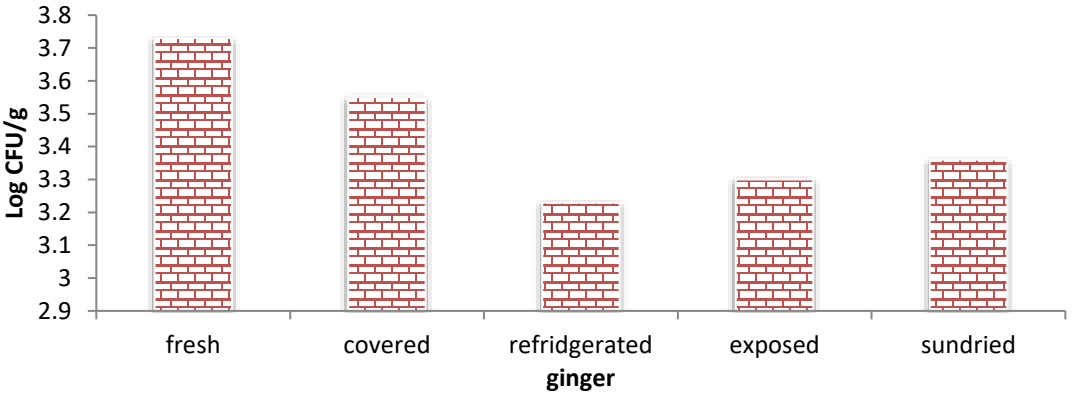

Fig 19 Bar chart of the total coliform count of ginger spice at different storage conditions

A total of 104 isolates were recovered from all spice samples at all storage condition.the highest number of isolates were recovered from garlic samples (26) while the least was recovered from uziza samples (15).

Table 1: Number of bacteria isolates recovered from all samples at all storage condition

Samples	***Staphylococcus* spp**	***Bacillus* sp**	***Micrococcus***	***Citrobacter***	***Escherichia* spp**	***Proteus spp***	***Serretia* spp**	**Total**
Uda	5	6	5	3	0	0	0	19
Nutmeg	4	7	5	0	0	0	0	16
Uziza	5	7	5	0	0	0	0	17
Garlic	5	10	5	0	0	0	4	24
Ginger	5	10	5	1	2	4	0	27
Total	24	40	25	4	2	4	4	103

Table .2: Number of fungi n isolates recovered from all samples at all storage condition

Samples	*Aspergillus* sp	*Penicillum* sp	*Sacchromyces* Sp	*Rhizopus* sp	*Fusarium* sp	Total
Uda	5	3	2	2	0	12
Nutmeg	5	3	4	2	0	14
Uziza	5	3	2	3	0	13
Garlic	5	5	3	4	2	19
Ginger	5	5	3	2	2	17
Toal	25	19	14	13	4	75

4. DISCUSSION

Studies in the past decade confirm that the growth of both gram-positive and –negative food borne bacteria, yeast and mold can be inhibited by spices. Nwaiwu and Imo (1999) have screened 3 Nigerian species; “Ehuru” (African nutmeg-*Monodara myristica*); “Uziza” (*Piper guineense*) and “Uda” (*Xylopia aethiopica*).

This present study reports the effect of Storage condition on microflora of Ginger, Garlic, Uda, Nutmeg and Uziza and the bacteria isolated were *Bacillus* (38.4%), *Micrcoccus* (24.0%), *Staphylococcus* (23.07%) were the most dominant genera, appearing in all spices samples in the different storage conditions. *Citrobacter* (3.84%) was found only on fresh, exposed, sun dried Uda and fresh ginger samples. *Escherichia* (1.9%) was isolated only on fresh and refrigerated ginger samples. *Serretia* (3.8%) appear only on fresh, exposed, covered and refrigerated ginger. *Proteus* (2.8%) was only recovered from fresh, exposed and covered garlic

samples.Amongst the 77 fungi isolates recovered from all samples, were 7 genera of fungi which include: *Aspergillus*, *Saccharomyces*, *Rhizophus*, *Penicillium* and *Fusarium* .

From this present study the TVC, staphylococcal count and coliform counts disagrees with results obtained by Shamsudeen 2009 which recorded a high coliform counts for all spice samples, high staphylococcal count (up to 10^7) and a high TVC of 10^8cfu/g (Shamsudeen, 2009). The result from this research differs from those obtained by Baxter and Holzapel (1982), Bhat et al., (1987), McKee(1995) Banarjee 2003 . This study corroborates with the findings obtained by Debs-Louka et al 2013 and Salari et al., (2012) having TVC within the range of 10^5 – 10^6 CFU/g of spices. The presence of bacteria and fungi may be due to poor storage and or handling (Asta,1999). Some of the organisms isolated have been implicated as causative agents of gastroenteritis (Nester *et al.,* 2001). However, the fungi result obtained correlated with those obtained by Shamsudeen (2009), but disagrees with those obtained by Salari *et al*., (2012). Shamsudeen (2009) obtained a TFC of 10^3 to 10^4 CFU/ml while those of Salari *et al.,* ranged from 10^4 – 10^5CFU/g.The acceptable standard for TVC, TFC and coliform count for spice according to FAO and the Codex Code of Hygiene practice (1995) are 10^5 – 10^6, 10^3 – 10^4, 10^3 respectively. 0/20g for *S. aureus*, 0/20g for *Bacillus cereus* and 0/20g for *E. coli* .

From the above standards it would be stated that all samples used in this research, although they have relatively high bacterial count, meet standards and are internationally acceptable. However the presence of *E.coli* in ginger samples , *E.coli* indicates possible contamination from a faecal source, *Staphylococcus* in food is enough to produce one microgram of enterotoxin enough to cause food intoxication and as such possible source of Staphylococcal contamination should be avoidedhe incidence of *Bacillus spp* is indicative of environmental contamination, which could have resulted from exposure of the spice to air or contact of utensils used with soil (Graven *etal.,* 1975). *Bacillus* in food causes intoxication and is capable of causing non gastrointestinal infection. *S.aureus* in the sample is indicative of human contamination after processing. The organism is associated with enterotoxin characterized by short incubation period, violent nausea, vomiting and diarrhea.The most frequent cause of microbiological contamination of spices was the finding of total number of microorganisms. Such finding was expected considering that most of the species were plants that may be contaminated from the ground by different microorganisms during its growth in the field, or by animal feces, and later by inadequate storage. Finding of moulds in spices as the second cause of contamination is the outcome of inadequate drying and storage in storehouses, where due to the increased humidity, moulds developed. . The presence of *A. species* in the spices might be due to air contamination (*Marcus etal.,* 1997)

The present of moulds may represent a spoilage problem or a potential public health problem. Toxigenic fungi may produce toxins under certain conditions. Since spices are soil-borne and sporeare ubiquitous in soil, the presence of large numbers of bacterial or mold spores can be expected.Spice is very important for aroma and increased palatability of our food. spices may even serve as source of contamination of processed product. Price and Schweigert, (1971), reported that unless spices are treated to reduce their microbial content, they may add high numbers and undesirable kind of organisms to food in which they are used. However, spice

with respect to choice of method of storage can cause contamination of food which could lead to microbial contamination, or early spoilage of our favourite spices.

From this research the method best for preserving various spices is by sun drying. One of the oldest forms of preservation of foods is drying. Furthermore, drying is essential for agricultural crops in developing countries (Eze and Agbo, 2011) in order to minimize spoilage and decay, improve shelf-life of the crop as well as minimize economic loss to farmers. The primary objective of drying is the removal of water from foods, which microorganisms require for growth,resulting in a more shelf stable, smaller and lighter food. Reduction in moisture discourages growth of spoilage or pathogenic microorganisms (Boyer and Huff, 2008). Furthermore, drying of herbs and spices is also used generally to extend the shelf-life of the resulting dried products.There are several methods of drying including sun or solar, onventional, microwave oven and the use of food dehydrator. Solar energy is a result of thermonuclear reactions of fusion from "hydrogen"into "helium" taking place in the sun (Balakrishnan *et al*., 2012), further, it is a direct transformation ofsolar UV light rays from sunlight to heat to infrared light rays, which causes the water, fat and protein molecules to vibrate and heat up (Diffey, 2002). There are different forms of solar drying, namely:traditional, solar box and sun oven. Traditional sun drying is low/no cost and crops are exposed to the environment. It is the preferred choice of drying for farmers in developing countries because of finance. This method of drying however is with many challenges including unpredictable weather, infestation of insects, pests and microbial contamination (kechucwu, 2010), and it poses a food safety problem for consumers. Solar box is a box fabricated with wood and glass cover of varying sizes while sun oven is a box with reflective panels. Food is placed in a pan painted black in the box. Both solar box and sun oven are left outside to absorb energy from the sun and are safer alternatives to the traditional concrete floor, open air drying and more beneficial.

REFERENCES

Debs-Louka E, El Zouki J, Dabboussi F. 2013. Assessment of the microbiological quality and safety of common spices and herbs sold in Lebanon. Journal Food Nutrition and Disorder.;2(4):1–6

Shamsuddeen U. 2009.Microbiological quality of spices used in the production of kilishi,a traditionally dried and grilled meatproduct. Bayero Pure Applied Science,2:66–9

Bhat R, Geeta H, Kulkarni PR. 1987. Microbial profile of cumin seeds and chili powder sold in retail shops in the city of Bombay. J. Food Protect., 50: 418-41

Price, J. F. and Schweigert B. S. 1971.The science of meat and meat products. 2nd Edition Published by W. H. Freeman and CompanyPp 289.

McKee, L. H. 1995. Microbial Contaminatio of Spices and Herbs: A Review. L.W. T., 28: 1–11.

Banarjee M, Sarkar PK. 2003. Microbiological quality of some retail spices in India. Food Res. Int., 36: 469-474.

Baxter R, Holzapfel WH. 1982. A microbial investigation of selected spices, herbs, and additives in South Africa. J. Food Sci., 47: 570578.

Diffey, B.L. 2002. Sources and measurement of ultraviolet radiation. Methods 4-13.

Ekechukwu, V.O. 2010. Solar Drying Technology: An overview Paper. Presented at FUTO. Alternative Energy Conference, Federal University of Technology Owerri

Graven; Mackel PC; N. B. Brain. 1975. International outbreak of Salmonella East borne infection traced to contaminated spices, 728-730.

Nestar EW, Anderson DG, Roberts CE,Peorsal NN, Nester MT. 2001. Food Microbiology Spectrum Book Ltd., Ibadan, Nigeria.

Marcus, M.J, Donald NW, Richard AR. 1997. Microbiology for the health sciences. 4th edition. Prentice Hall Upper Saddle River, New Jersey.

Boyer, R. and Huff, K. 2008. Using Dehydration to Preserve Fruits, Vegetables, and Meats. Virginia Cooperative Extension Publication 348-597

Eze J.I. and Akubor P.I. 2012. Effect of drying methods and storage on the Physicochemical properties of Okra. Journal of Food Process Technology 3(88): 173- 177.

Balakrishnan, M., Claude, A. and Arun Kumar, D.R. 2012. Engineering, design and fabrication of a solar cooker with parabolic concentrator for heating, drying and cooking purposes. Archives of Applied Science Research 4(4): 1636—1649

Sangwan, A., Kawatra, A. and Sehgal, S. 2012. Nutritionalcomposition of ginger powder prepared using various drying methods. Journal of Food Science Technology 51(9): 2260-2262

Salari B, Habibi-Najafi, Boroushaki MT,Mortazavi SA, Fathi Najafi M. 2012. Assessment of the microbiological quality and mycotoxin contamination of Iranian red pepper spice. J. Agr.Sci. Tech.14: 1511-1521.

Shamsuddeen, U. and Ameh, J. B. 2008. Survey on the possible critical control points in kilishi (a traditional dried and grilled meat snack) produced in kano. International Journal of Bioscience. 3(2): 34-38.

Cite this article:
Omorodion Nnenna JP, "Effect of Different Storage Conditions on the Microbial Profile of some commonly used Spices in Nigeria," Journal of Multidimensional Research and Review (JMRR), Vol.1, Iss.2, pp.91-104, 2020