



Microflora of Three Dehydrated Vegetables

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Authors' contributions

This work was carried out in collaboration between all authors. This is part of author AAA dissertation and was supervised by author LOA at Olabisi Onabanjo University, Ogun State, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Consumption of fresh vegetables is very common in Nigeria. One of the methods of increasing the availability of vegetables all year round is drying. There is need to determine the microbial quality/safety of the dehydrated vegetables in order to determine the risk of food borne diseases. The microorganisms associated with three dehydrated vegetables (bitter leaf, bell pepper and okra) were isolated, identified and enumerated. The moisture contents of the vegetables were determined and the effects of varying temperature levels on growth of microbial isolates recovered from the samples were studied. A total of nine bacteria, namely: *Staphylococcus aureus*, *Acinetobacter iwoffii*, *Corynebacterium* sp., *Bacillus pumilus*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Flavobacterium* sp., *Bacillus* sp., *Micrococcus kristianae* and eleven fungi: *Aspergillus niger*, *A. flavus*, *Aspergillus* spp., *Penicillium* spp., *Cladosporium* sp., *Fusarium* spp. were isolated. The mean for total colony forming units (cfu/g) for bacteria were 2.1×10^7 cfu/g, 6.1×10^5 cfu/g, 2.2×10^6 cfu/g for bell pepper, bitter leaf and okra, respectively while the mean for total colony forming unit (cfu/g) for fungi were highest (1.3×10^6 cfu/g) in bell pepper while bitter leaf recorded the least (7×10^3 cfu/g) mean for total colony unit for fungi. The mean for percentage (%) moisture content ranged between 16.6-25.8%. The optimum growth was recorded for all the bacteria and fungi at 37°C and 30°C; nearly all the isolates had their growth retarded at 45°C. The recovery of several harmful microorganisms in this study suggest the need for proper handling of vegetables during processing and storage to minimize microbial contamination in order to protect consumers' health.

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1. INTRODUCTION

Vegetable consumption provides a valuable contribution to the nutrition of rural and urban populations in West Africa. Numerous studies have recorded uses and consumption patterns of vegetables [1], but few reports have been published on dried vegetables. Drying of foods is practiced in Africa to make the products more durable and preserve them for food insecure periods. The infection of dehydrated products could be from the inhabited microbes and other contaminants in the product, or as a result of unhygienic condition that takes place during the treatment (drying process). Improper handling and storage could possibly enhance its spoilage [2,3]. Crops or products that are susceptible to fungal growth can also be contaminated with mycotoxins [4]. Mycotoxins are hazardous to consumers' health and affect food quality leading to economic losses including loss of commercial value [5].

Foods, microorganisms and humans have had a long and interesting association that developed long before recorded history. Foods are of nutritional value to those who consume them but on the other hand are ideal culture media for microbial growth. The presence and availability of water also affect the ability of microorganisms to colonize foods. However, by simply drying a food one can control or eliminate spoilage process.

Contaminated foods can transmit a wide range of diseases to humans. In a food infection, the food transfers the pathogen to the consumer, in whom the pathogen grows and cause disease. With food intoxication, the microorganisms grow in the food and produce toxins that can then affect the consumer. Foods that are consumed as raw, pose a risk of disease transmission if care is not taken in production, storage and transportation. Without adequate care, major disease outbreaks can occur, because foods are transported around the globe in extremely short times.

Vegetables and fruits are important aspect of the human diet in Nigeria because of their nutritional value [6]. However they are usually in short supply during dry seasons because they are perishable crops, which deteriorate within a few days after harvest (which occurs mainly in the rainy season). Aliyu and Sambo [7] reported that preserving vegetables in their fresh state for months has been a problem yet to be solved in the country. A very common method of preservation of these agricultural crops is to dry them in order to conserve the perishable fruits, reduce storage volume and extend their shelf life beyond the few weeks when they are in season [8]. Drying can either be done by traditional sun - drying or industrially through the use of solar dryers or hot air drying [9].

Dehydrated foods are probably never sterile. In this respect they differ from canned foods. Therefore, it is of utmost importance to prevent entrance of organisms which are capable of producing food poisoning into such foods. This applies especially to the toxigenic forms of *Clostridium botulinum* and certain strains of *Staphylococcus aureus*. Dehydrated foods should also be free from certain intestinal forms likely to be pathogenic to man when taken by mouth, e.g member of the genera *Salmonella* and *Shigella*. The bacteria count of dried foods should be reasonably low to avoid decomposition or development of undesirable flavours during the period of reconstitution.

To prevent the formation of bacteria toxins or the development of organisms pathogenic to man, the food should be dried at a temperature where significant growth is unlikely to occur. Dehydration of foods is a valuable procedure for several important reasons [10]. Dried foods

may be easily preserved for future use. This means that certain foods may be utilized over long periods of time rather than for only a short season of the year. Dehydration greatly reduces the bulk of a product, conserves space and facilitates handling. This is a decided advantage from the stand point of transportation costs. Most of the dehydrated products, if properly prepared are very good substitutes for fresh foods, being detected from the normal product with difficulty. Sometimes dehydrated foods become infested with insects owing to improper packaging or handling. Sometimes dried foods become moistened, creating favorable condition for the growth of bacteria, yeast and mould [10].

The purpose of the study is to determine the microbiology of three dehydrated vegetables (bitter leaf, bell pepper and okra) which includes: isolation and identification of the microorganisms associated with three dehydrated vegetables (okra, bitter leaf and bell pepper), evaluating the bacterial and fungal loads of the samples, assessing the moisture content levels of the samples and to determine the growth of the associated organisms under varying temperature levels.

2. MATERIALS AND METHODS

2.1 Sampling

Twenty five samples each of the dehydrated vegetables (bell pepper, okra, and bitter leaf) were purchased from the local markets (Iperu, Ilisan, Irolu, Ogere and Ikenne) in Ikenne Local Government Area, Ogun State, Nigeria.

2.2 Moisture Content Analysis

Five- gram- portion of each of the samples (bell pepper, okra and bitter leaf) was placed in Petri dishes which were then placed in an oven (Gallenkomp, England) at temperature of 100⁰C. The samples were weighed (Using Gallenkomp balance, England) every hour until three consecutive constant weights were obtained. The new weights of the samples were measured and the percentage moisture content was calculated using the formula.

$$\% \text{ moisture} = \frac{\text{initial weight of sample} - \text{final weight of sample}}{\text{Initial weight of sample}} \times 100$$

Where: initial weight of sample = weight of sample before placing in oven.

Final weight of sample = weight of the sample after heating in the oven.

2.3 Isolation

One gram-portion of each sample was weighed and mashed in a laboratory mortar. The mashed sample was added to 9 ml of peptone water (Oxoid, Cambridge, UK) and shaken vigorously and serial dilution was carried out up to the 10⁻⁶ dilution factor. One milliliter of each to dilution factors were poured aseptically into corresponding Petri plates of sterile ten nutrient agar (Oxoid, Cambridge, UK) cooled to about 43C and then immediately swirled gently for about 10 seconds before setting. The same procedure was repeated for Potato Dextrose Agar (Oxoid, Cambridge, UK) supplement with 250 mg/L of chloramphenicol (Bulicol, Jiangsu, China) and streptomycin (Bulicol, Jiangsu, China) to isolate fungi. Duplicate of inoculated plates were made and all nutrient agar plates were incubated at 37⁰C for 24hrs while PDA plates were incubated at 30⁰C for 5 days.

2.4 Characterization and Identification of Organisms

Characterization and identification of bacteria species involved the following: Grams staining and biochemical/physiological examination of isolates such as patterns of sugar fermentation, assimilation of sugars, and production of certain enzymes. This was achieved by sub culturing mixed bacterial cultures from nutrients agar plates into freshly prepared sterile nutrient agar plates to obtain pure cultures of each isolate. The stock cultures were prepared by inoculating from pure slants. These slants were incubated at 37°C for 24 hrs after which they were stored at 4°C.

Pure cultures of fungal isolates were also made by inoculating characterized isolates into potato dextrose agar slants. These were incubated at 30°C for 5days and stored at 4°C. The fungal species were identified by macroscopic and microscopic - Photomicrograph (using Olympus microscope, Tokyo, Japan) observations.

2.4.1 Identifications of bacterial isolates

Pure cultures of the isolates were subjected to various biochemical tests as described by [11] to determine the probable identity of the bacteria. The result of each test was recorded and the probable identity of the isolate was determined by the use of Bergey's Manual of Determine Bacteriology.

2.4.2 Identification of fungal isolates

Pure cultures of fungi isolates were established using single sporing method [12]. Identification was based on colonia morphology (colour, mycelia texture growth pattern, reverse plate pigmentation, etc.) and microscopy which include hyphae structure and spore appearances (type, colour, shape, etc.) [13].

2.5 Effect of Varying Temperature Levels on Growth of Isolates

2.5.1 Effect of varying temperature levels on bacterial growth

The test was carried out by inoculating freshly prepared sterile nutrient broth (Oxoid, Cambridge, UK) with each of the bacterial isolates and incubated at 4°C, 30°C, 37°C and 45°C for 24 hrs. The turbidity of the broth cultures after incubation were observed and compared with uninoculated nutrient broth.

2.5.2 Effect of varying temperature levels on fungal growth

The test was carried out by inoculating aseptically freshly prepared potato dextrose agar using 5mm cork borer discs of the 7-day- old culture of the isolates, incubation was done for 6 days at 30°C and 45°C. The diameters of the growth at 30°C and 45°C were measured, this test was carried out five times and the mean values were recorded.

3. RESULTS

3.1 Moisture Content Analysis

Moisture content of the samples ranged between 16.6-25.8%. Bell pepper had the highest (25.8%) moisture content, while bitter leaf recorded the least (16.6 %,) as illustrated in Table 1.

3.2 Total Colony Forming Units of Microbial Isolates

The total colony forming units of the bacteria for the dehydrated bell pepper samples ranged between 1.4×10^7 and 2.9×10^7 cfu/g with the mean value of 2.1×10^7 cfu/g, while bitter leaf gave total colony forming units ranging from 5.7×10^5 to 6.8×10^5 cfu/g with the mean value of 6.1×10^5 cfu/g. For okra, the value ranged between 1.6×10^6 and 2.9×10^6 cfu/g with the mean value of 2.2×10^6 cfu/g.

The total colony forming units of the fungi ranged between 8.5×10^5 and 9.8×10^5 cfu/g for okra, 1.1×10^6 and 1.5×10^6 cfu/g for bell pepper, while for bitter leaf it ranged between 6.7×10^3 and 7.3×10^3 cfu/g. The mean value of total cfu/g for fungi was highest (1.3×10^6) in bell pepper while bitter leaf recorded the least value (7×10^3) as illustrated in Table 1

Table 1. The mean and standard deviation for percent (%) moisture content and total colony forming units (cfu/g) of the bacteria and fungi recovered from the dehydrated samples

	BPP (Bell pepper)	OKR (Okra)	BLF (Bitter leaf)
% Moisture Content	25.8±1.8	18.7 ±0.9	16.6 ±0.8
Total colony forming units for bacteria.(cfu/g)	$2.1 \times 10^7 \pm 0.4 \times 10^7$	$2.2 \times 10^6 \pm 0.4 \times 10^6$	$6.1 \times 10^5 \pm 0.4 \times 10^5$
Total colony forming units for fungi.(cfu/g)	$1.3 \times 10^6 \pm 0.1 \times 10^6$	$9.0 \times 10^5 \pm 0.4 \times 10^5$	$7 \times 10^3 \pm 0.2 \times 10^3$

± Standard deviation

3.3 Identification of microorganisms

3.2.1 Identification of bacterial isolates

Characterization of the bacteria was carried out by Gram staining reaction and biochemical tests. The results showed that *Staphylococcus aureus*, *Acinetobacter iwoffii*, *Corynebacterium* sp., *Bacillus pumilus*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Flavobacterium* sp., *Bacillus* sp., and *Micrococcus kristianae* were bacteria isolated from the dehydrated bitter leaf, bell pepper and okra samples, as illustrated in Table 2.

Table 2. Gram's reaction and biochemical characteristics of bacterial isolates

Lab code	Grams reaction*	Catalase	Oxidase	Indole	Methyl red	Voges proskauer	Citrate	Urease	Starch hydrolysis	Coagulase	Glucose	Xylose	Sucrose	Lactose	Maltose	Galactose	Probable identity
OKR ₁	GPC	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	<i>S. aureus</i>
OKR ₂	GNR	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	<i>A. ivoffi</i>
OKR ₃	GPR	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	<i>Corynebacterium sp</i>
OKR ₄	GPR	+	-	-	-	-	+	+	-	-	+	-	+	-	+	-	<i>Bacillus pumilus</i>
BLF ₁	GPC	+	-	-	-	-	-	-	-	-	+	+	+	-	+	-	<i>Micrococcus sp.</i>

BLF ₂	GNR	+	+	-	-	-	+	-	-	-	+	-	+	-	+	-	<i>P.auregin</i> <i>osa</i>
BLF ₅	GNR	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-	<i>Flavobacteriu</i> <i>m sp.</i>
BLF ₆	GPR	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	<i>Bacillus</i> <i>sp.</i>
BPP ₁	GPC	+	+	-	-	-	+	+	-	+	+	+	-	+	-	-	<i>Micrococc</i> <i>us</i> <i>kristiana</i>

KEY:GPC- Gram positive cocci; GNR-Gram negative rod; GPR- Gram positive rod.
OKR- Okra, BLF- Bitter Leaf, BPP- Bell Pepper.

3.2.2 Identification of fungal isolates

The fungal isolates were identified by microscopy-Photomicrograph (Olympus microscope) and morphology. A total of eleven fungal species were isolated and identified. They include, *Aspergillus niger* as shown in Fig. 1, *A. flavus*, *Aspergillus* sp.1, *Aspergillus* sp. 2 as shown in Fig. 2, *Aspergillus* sp. 3, *Penicillium* sp. 1, *Penicillium* sp.2, *Penicillium* sp. 3, *Cladosporium* sp., *Fusarium* sp.1, *Fusarium* sp. 2 as shown in Fig. 3.

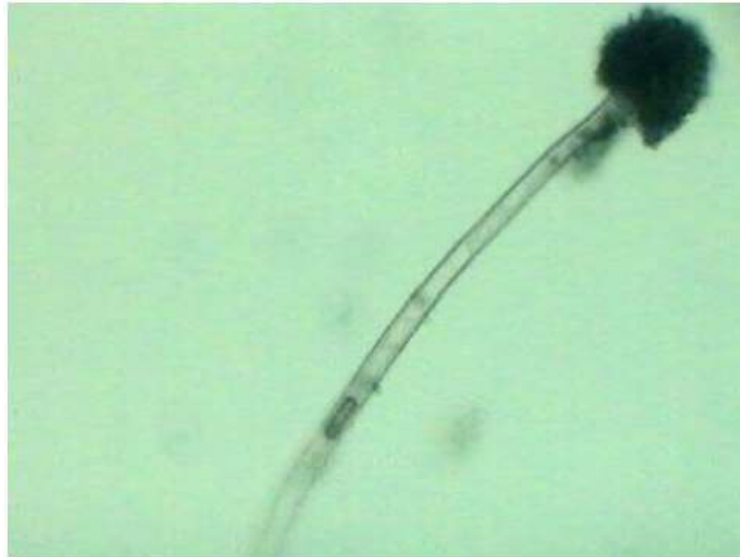


Fig. 1. Photomicrograph of *Aspergillus niger*

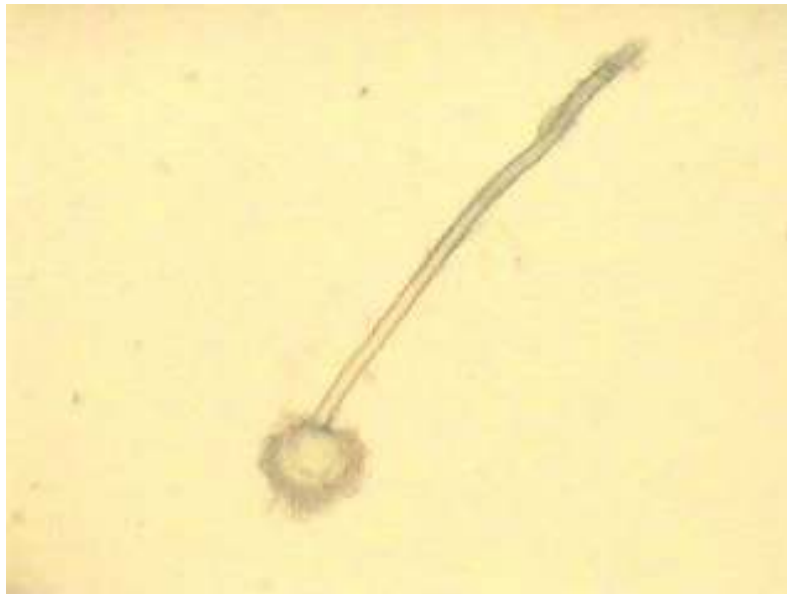


Fig. 2. Photomicrograph of *Aspergillus* sp



Fig. 3. Photomicrograph of *Fusarium* sp

3.3 Percent Incidence of Species of Bacteria and Fungi Isolated from Dehydrated Vegetable Samples

The isolated organisms and their percent incidence of occurrence are summarized in Tables 3 and 4. Altogether twelve (12) organisms: four (4) bacteria species and eight (8) fungi species were recovered from the twenty five (25) samples of dehydrated bitter leaf analyzed. *Pseudomonas aeruginosa* (15), *Flavobacterium* sp. (11), *Bacillus* sp. (10), *Micrococcus* sp. (13), *Aspergillus niger* (21), *Penicillium* sp.1 (19), *Penicillium* sp.2 (22), *Penicillium* sp.3 (20), *Aspergillus* sp.1 (25), *Aspergillus* sp.2 (24), *Aspergillus* sp.3 (22) and *A. flavus* (23) with percent incidence of 60,44, 40, 52, 84, 76, 88, 80, 100, 96, 88, and 92 respectively.

A total of four microorganisms: one bacterium, namely *Micrococcus kristianae* (09) and three fungi, *Cladosporium* sp.(14), *Aspergillus* sp.1(25) and *Aspergillus* sp.2(21) were recovered from twenty five samples of dehydrated bell pepper investigated with percent incidence of 36, 56, 100 and 84, respectively.

From the twenty five samples of dehydrated okra investigated, *Staphylococcus aureus*, *Acinetobacter iwoffii*, *Corynebacterium* sp., *Bacillus pumilus*, *Penicillium* sp.1, *Penicillium* sp.2, *Penicillium* sp.3, *Fusarium* sp.1 and *Fusarium* sp.2, were isolated. *Corynebacterium* sp. recorded the least (40) percent incidence while *Penicillium* sp.3 had the highest (92).

Table 3 Percent (%) incidence of bacteria isolated from dehydrated vegetable samples

	<i>S.aureus</i>	<i>Acinetobacter iwoffii</i>	<i>Corynebacterium sp.</i>	<i>Bacillus pumilus</i>	<i>Micrococcus kristianae</i>	<i>P.aeruginosa</i>	<i>Flavobacterium sp.</i>	<i>Bacillus sp.</i>	<i>Micrococcus sp.</i>
BLF	-	-	-	-	-	60	44	40	52
BPP	-	-	-	-	36	-	-	-	-
OKR	52	44	40	60	-	-	-	-	-

BLF: Bitter Leaf, BPP: Bell Pepper, OKR: Okra

Table 4. Percent (%) incidence of fungi isolated from dehydrated vegetable samples

	<i>A.niger</i>	<i>A. flavus</i>	<i>Aspergillus sp.1</i>	<i>Aspergillus sp.2</i>	<i>Aspergillus sp.3</i>	<i>Penicillium m sp.1</i>	<i>Penicillium m sp.2</i>	<i>Penicillium m sp.3</i>	<i>Cladosporium sp.</i>	<i>Fusarium sp.1</i>	<i>Fusarium sp.2</i>
BLF	84	92	100	96	88	76	88	80	-	-	-
BPP	-	-	100	84	-	-	-	-	56	-	-
OKR	-	-	-	-	-	80	84	92	-	72	84

BLF: Bitter Leaf, BPP: Bell Pepper, OKR: Okra.

Table 5. Effect of varying temperature levels on the visible growth of isolated bacteria

Bacterial isolates	Temperature °C			
	4	30	37	45
<i>Staphylococcus aureus</i>	-	+	++	-
<i>Acinetobacter iwoffii</i>	-	+	++	-
<i>Corynebacterium sp.</i>	-	+	++	++
<i>Bacillus pumilus</i>	-	+	++	-
<i>Micrococcus sp.</i>	-	+	++	+
<i>Pseudomonas aeruginosa</i>	-	+	++	-
<i>Flavobacterium sp.</i>	-	+	++	-
<i>Bacillus sp.</i>	-	+	++	+
<i>Micrococcus kristianae</i>	-	+	++	-

Decoder: - =No growth; += slight growth; ++= Moderate growth.

Table 6. Effect of temperature on the radial growth (diameter/mm) of fungal isolates

Fungal isolates	Temperature/diameter (°C/mm)	
	30	45
<i>Penicillium</i> sp.1	13.4	0
<i>Fusarium</i> sp.	37.0	0
<i>Penicillium</i> sp.2	19.6	0
<i>Aspergillus niger</i>	18.2	0
<i>Penicillium</i> sp.3	12.8	0
<i>Aspergillus</i> sp.1	12.6	0
<i>A. flavus</i>	22.6	0
<i>Cladosporium</i> sp.	15.0	0
<i>Aspergillus</i> sp.2	19.8	0

Each figure is an average of values from five replicates

3.4 Discussion

The dehydrated bitter leaf had the lowest mean value of percent moisture content (16.6%) followed by the okra (18.7%) and the highest was recorded for bell pepper (25.8%). The most prevalent fungi were *Aspergillus* spp. which supports the findings of Ravi Kiran et al. [14]. The climatic conditions in West Africa are favorable for fungal development with high relative humidity [15], high temperature [16] and little aeration [17], all conditions that accelerate fungal and mycotoxin development. In addition, in African markets, movement of people and vehicles, unloading and loading of trucks in confined spaces are common; as a result, the air in these markets abounds with dust and potentially microbial spores. The aforementioned climatic and environmental conditions are highly favorable for the propagation of fungi, especially the genera *Aspergillus*, *Penicillium*, *Mucor*, *Rhizomucor* and *Rhizopus* that produce and release a lot of spores [18].

Staphylococcus aureus is one of the bacteria isolated from okra. Staphylococcal food poisoning is the major type of food intoxication in the United States. It is caused by ingestion of improperly stored or cooked food in which *S. aureus* has grown. It is found in the nasal passages and on the skin of humans and other mammals worldwide; from these sources it can readily enter food. It is very resistant to heat, drying and radiation. If *S. aureus* was allowed to incubate in certain foods, they produce heat-stable enterotoxin that renders the food dangerous even though it appears normal. Once the bacterium or organism has produced the toxin, the food can be extensively and properly cooked, killing the cells of the bacteria but without destroying the toxin [19]. *Micrococcus* sp. was isolated from bitter leaf and bell pepper although the organism is not pathogenic; they are primarily on mammalian skin and in soil.

In considering the effect of temperature, all the bacterial isolates flourished best at 37°C. No growth was observed at 4°C and at 45°C for many of the bacteria as illustrated in Table 5. The species of *Bacillus* grew at elevated temperature (45°C). Table 6 shows that all the fungi had optimum growth at 30°C. All molds were affected by the elevated temperature [20]. This implies that the dehydrated vegetables after being purchased from the local market could be stored in the refrigerator or sun-dry in order to inhibit the microbial growth and not to be stored at room temperature (28-30°C).

Processing and packaging methods can have an effect on microbial contamination and levels of infestation. Dried vegetables and spices stored in gunny bags and on bare ground had significantly higher incidence of fungi compared to those stored in wooden boxes, metal or glass containers [21]. The same author also noted that fungal infestation is compounded by insect damage in the field and during transportation. The relationship of fungal infestation and insect damage is also mentioned by [22]. Another source of fungal infestation can be the result of inappropriate handling and storage methods, often associated with poor hygiene [23]. [24] Postulated that “contamination of foodstuffs with spoilage fungi was the result of natural extraneous pollution with dust particles containing spores” during storage. This could probably have been one of the factors that lead to the contamination of the dehydrated vegetables analyzed since all of them were harvested, processed, stored and marketed under ambient conditions with little protection from dust and fungal spores.

Several abiotic factors influence the growth of moulds on dried vegetables; they are relative humidity and temperature together with the moisture content of the product and storage conditions [25]. These factors are known to facilitate the development of the most prevalent fungi observed. Most mycotoxin producing fungal species grow at the lower limits of moisture content ranging from 12 to 13% [26]. Hall (1986) reported that the moisture content of 4.2% is considered to be safe enough for storage of vegetables and fruits [27]. Prolonged storage in poorly ventilated structures or containers, increase moisture content of the commodity, rendering the stored products more susceptible to mould growth and toxin production [21] and potentially the infestation with storage insects.

This study showed that dehydrated vegetables from the local markets (Iperu, Ilisan, Irolu, Ogere and Ikenne) in Ikenne Local Government Area, Ogun State, Nigeria were contaminated with fungi that can potentially lead to mycotoxin development. It was observed that dried vegetable is a good substrate for mycotoxin producing fungi and these products are exposed to environmental conditions that favor fungal development and mycotoxin formation. It has been reported that chronic ingestion of foods that are contaminated with mycotoxins can lead to much greater health risks than previously perceived [28].

On the other hand there are certain technologies that could reduce microbial contamination in the surveyed vegetables. These include improved processing, packaging, storage and handling practices. Many of these options need to be developed and/or adapted to African conditions. Also consumers in the community need to be informed about these potential options in order to improve product quality and reduce microbial contamination. Ideally such products should pass strict quality control inspection before being marketed to consumers.

4. CONCLUSION

A variety of bacteria and moulds were isolated as contaminants from the selected dehydrated vegetables of which some can cause food poisoning e.g, *Staphylococcus aureus* in this study. The environmental conditions such as relative humidity, temperature, pH, etc, have great influence on the survival of microorganisms isolated. Furthermore, microbial contamination could be reduced/prevented if the vegetables are processed, packaged and stored properly.

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COMPETING INTERESTS

Authors have declared that no competing interests exist but to educate the public.

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