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Contamination of Retailed Yam Products at Two Popular Markets in Ibadan with *Pseudomonas Spp*, *Enterobacter Spp* and *Flavobacterium Spp*

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ABSTRACT

Contamination of commercially processed and marketed yam products by pathogenic microorganisms can have adverse and chronic effects on health if not approached properly. Different types of bacteria were isolated using standard methods to identify yam flour samples obtained from the Oja Oba and Bodija markets. They are Bacillus cereus, Bacillus subtilis, Pseudomonas spp, Flavobacterium spp, Enterobacter spp. Associated fungi like A. flavus, A. niger, P. oxalicum, R. stolonifer were found. The total microbial counts recorded in first and second analysis ranges 1.4 x 10^4 to 6 x 10^4 and 3.6 x 10^4 to 9 x 10^3 respectively in the Bodija market while in the Oja Oba market; The first and second analyzes range from 2.9 x 10^4 to 1.7 x 10^4 and 8.1 x 10^4 to 1.5 x 10^4, respectively. Including isolations of bacteria and the percentage of their occurrence; In Bodija market; Bacillus subtilis 30%, Bacillus cereus 20%, Pseudomonas sp. 35%, Flavobacterium sp. 5%, Enterobacter sp. 10% while Oja Oba; Bacillus subtilis 35%, Pseudomonas sp. 30%, Flavobacterium sp. 10%, Bacillus cereus 20% and Enterobacter sp. 5%. The control samples produced did not contain Pseudomonas spp, Enterobacter spp. or Flavobacterium spp. population compared to all collected market samples. These bacteria are gram-negative bacteria, they are everywhere. Pollution level of Pseudomonas spp. they do not differ significantly in different areas of the market sites regardless of market status and standards, and they have the same opportunity to introduce perishable or spoilage bacteria such as toxin producing bacteria; Pseudomonas spp., Flavobacterium spp. and Enterobacter spp if they neglect proper food handling activities from yam to food which can cause deleterious effect on human health when consumed.

Keywords
Contamination, Pathogenic microorganisms, Yam, Health effect, Food safety.

Introduction
Yam (*Dioscorea* spp.) is a tuber crop with several edible species in the family Dioscoreaceae [1]. Yam is the highest produced tuber crop in the world with an average of 75 million tonnes produced from over 9 million hectares in 2021 [2]. West Africa is the highest world producer of yam (95% of world yam production), which is an important crop to the sociocultural and economic life of several farmers [2,3]. Yam is a profitable crop though with high production cost and it is majorly grown by small scale farmers and intercropped with cereals, legumes, and vegetables [4-7]. *Dioscorea rotundata* is the most important species in the yam production zone, however, water yam, *Dioscorea alata*, is the most widely distributed species in the world. Yam is the preferred staple food in West Africa, but demand is limited by losses in adequate production and storage.

Yam crops face stress from insect pests, bacteria, fungi and viral diseases as well as soil-borne nematodes associated with intensive cropping patterns [8]. *Pseudomonas* is a genus of gram-negative, non-spore forming, aerobic bacteria, belonging to the family Pseudomonadaceae, they have high genetic diversity and poor nutritional needs allowing them to survive in different environments, such as atmospheric dust, vegetation, water, and soil [9,10]. Additionally, these characteristics allow them to

survive on the utensils and equipment used in the dairy production chain, such as milking machines, pipelines, and bulk tanks [11]. Microorganisms are known to destroy the yam, thereby reducing the quality and quantity of the crop for consumption and while increasing cost from planting to post-harvest stage [12]. Microorganisms especially the pathogenic ones lead to reduced germination and plant vigor [13]. Degeneration of most crops is often associated with bacterial and fungal infections [14]. The presence of these pathogens in yam products can cross food items during food handling, which can have an adverse and chronic effect on health [15]. Therefore, this study evaluated the microbial contamination of different yam varieties in two markets in Ibadan, to determine the extent of contamination so has to create a premise for the development of control strategies from field, to store and to market.

Materials and Methods

Description of Study Site

Samples of dried yam were obtained in two markets, namely "Oja-Oba" and "Bodija" markets in Ibadan, both in the South western Oyo State. Oja-Oba market is located at 25^{0}7.22.25¨N latitude and 3.53.42°E longitude. Oja Oba is located along Ibadan South-West local Government Area of Oyo State and one of biggest food stuff market in Ibadan which composed of thousands of independent sellers who compete for sales of major food items like Yam and its different products, Maize, Cowpea or Beans, Rice, Vegetables, Palm and vegetable Oils, Cassava products, Onions, Tomatoes, Pepper, Millet, Sorghum, fruits etc. Bodija Market is an international market located at 7.4358°N latitude and 3.9192°E longitude along Ibadan North local government area of Oyo state. The market is designed in such a way that every farm produces namely Rice, Beans, Pepper, Yam, Garri, dried Maize, Groundnuts, Tomatoes etc. has its own row stalls, well arranged and lined. The market can also be described as a mixture of open space trading, concrete and wooden stalls. The both markets are two popular markets in Ibadan. The tissue culture unit of NACGRAB is involved in *in vitro* micropopagation of different crops.

Collection of samples

Samples of retailed dried yam were randomly obtained by purchasing it from the sellers at the two popular markets (figure 1). Three samples each of yam products of different yam types from different sources of supply were collected for a period of six (6) weeks (Table 1). The control samples were fresh samples collected in NACGRAB, peeled and dried using a drying machine. All the samples were grounded using laboratory blending machine.

Table 1: The native nomenclature for different yam tubers products being sold at the markets.

Yoruba name	Scientific name	English name
Ewura	*Dioscorea alata*	Water yam
Gbararo	*Dioscorea rotundata*	White yam
Kiayomo	*Dioscorea rotundata*	white yam
Ipokoro	*Dioscorea alata*	water yam
Kunube	*Dioscorea alata*	Water yam

Media Preparation and Sterilization

For this experiment, NBY (Nutrient Broth Yeast Agar) supplemented with 1ml of 1M $MgSO_4$ solution after autoclaving at 121°C for 15mins was used which support the growth of both bacterial and fungi [16,17].

Microbiological Analysis

The total possible microbial count of yam flour samples was determined by pour plate method [18] with modification. 1g of yam flour was taken and diluted with 9 ml of sterile distilled water. Serial dilution was performed to dilution factor 10^{-6}. The plates were left to gel and then incubated at 28°C for 24hr to 48hrs. At the end of each incubation period, the colonies were counted using colony counter, and subcultured by streaking on nutrient agar plates to obtain pure bacteria colonies and later stored in the refrigerator at 4°C for biochemical test and identification.

Result

In this study, the results obtained in the microbial analysis of dry white and water yam revealed different types of microorganisms. They were *Bacillus sp, Pseudomonas sp, Flavobacterium sp, Enterobacter sp, Rhizopus stolonifer, Aspergillus niger, Penicillium oxalicum, Mucor, Aspergillus flavus, Pythium*. In Bodija, the total viable bacterial count of first and second experiment ranges from 1.3 x 10^4 to 7 x 10^3 and 3.6 x 10^4 to 9 x 10^3 respectively (Table 2). The genera of the bacteria isolated and their percentage of occurrence include; *Bacillus subtilis* 30%, *Bacillus cereus* 20%, *Pseudomonas sp* 35%, *Enterobacter sp* 10% and *Flavobacteria sp* 5% (Figure 4) while in Oja Oba, the total bacterial count ranges from 2.9 x 10^4 to 1.7 x 10^4 and 8.1 x 10^4 to 1.5 x 10^4 (Table 2). The percentage of occurrence include; *Bacillus subtilis* 35%, *Pseudomonas sp* 30%, *Flavobacterium* 10%, *Bacillus cereus* 20% and *Enterobacter sp* 5% (Figure 4). The prepared control samples had low microorganism population and no *Pseudomonas spp, Enterobacter spp* or *Flavobacterium spp* population compared with all the collected market samples. Table 3, shows the result of different biochemical reaction test.

Discussion

Pseudomonas, Enterobacter and Flavobacterium has been associated with food spoilage, thereby decreasing the quality and quantity of the crop for intake and benefit from field to postharvest stage [19,20]. The deterioration of food is frequently related to microorganisms especially bacteria and fungi [21]. Pseudomonas is a genus of bacteria, which are known to produce toxins that can be harmful and generally associated with food spoilage [22,23]. The presence of those microorganisms in yam products contaminates the food product and causes cross-contamination of meals, when consumed by humans, it causes impairment of their fitness [21].

This study shows the contamination level of bacteria (*Pseudomonas sp* and *Flavobacterium spp* and *Enterobacter spp*) on yam product at famous markets in Ibadan. From the samples, exclusive species of bacteria and fungi had been intercepted for identity by the use of pour-plate methods. Some of those remoted micro-organisms

Table 2: Comparison in the Different Markets' Results of the first and second analysis of the Different Yam Samples.

Location	Samples	Total Viable Microbial Count		Microbes Isolated
		First Analysis	Second Analysis	Bacteria and fungi
BODIJA	Ewura (Water yam)	4.2×10^4	1.02×10^5	*Bacillus subtilis, Bacillus cereus, Pseudomonas sp, Rhizopus stolonifer, Penicillium oxalicum, Aspergillus flavus*
OJA OBA	Ewura (Water yam)	5.4×10^4	1.62×10^5	*Bacillus subtilis, Bacillus cereus, Pseudomonas sp, Rhizopus stolonifer, Penicillium oxalicum, Aspergillus niger, Flavobacterium spp*
BODIJA	Gbararo (White yam)	6×10^4	9.0×10^3	*Bacillus subtilis, Bacillus cereus, Aspergillus niger, Penicillium oxalicum, Pythium sp, Rhizopus stolonifer, Fusarium oxysporum, Aspergillus flavus*
OJA OBA	Gbararo (White yam)	1.7×10^4	1.5×10^4	*Aspergillus flavus, Rhizopus stolonifer, Penicillium oxalicum, Bacillus subtilis, Bacillus cereus, Aspergillus niger, Pseudomonas sp*
BODIJA	Ipokoro (White yam)	1.4×10^4	2.5×10^4	*Pseudomonas sp, Bacillus subtilis, Bacillus cereus, Rhizopus stolonifer, Aspergillus niger, Penicillium oxalicum, Aspergillus flavus, Enterobacter sp*
OJA OBA	Ipokoro (White yam)	2.9×10^4	8.1×10^4	*Bacillus subtilis, Bacillus cereus, Pseudomonas sp, Mucor, Aspergillus flavus, Rhizopus stolonifer, Aspergillus niger, Penicillium oxalicum*
BODIJA	Kiayomo (White yam)	9×10^3	3.6×10^4	*Bacillus subtilis, Bacillus cereus, Flavobacterium sp, Pseudomonas sp, Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Penicillium oxalicum*
OJA OBA	Kiayomo (White yam)	2.5×10^4	3.1×10^4	*Bacillus subtilis, Pseudomonas sp, Bacillus cereus, Penicillium oxalicum, Rhizopus stolonifer, Aspergillus niger*
BODIJA	Kunube (White yam)	5.9×10^4	2.56×10^5	*Pseudomonas sp, Bacillus subtilis, Bacillus cereus, Aspergillus niger, Penicillium oxalicum, Aspergillus flavus, Rhizopus stolonifer,*
OJA OBA	Kunube (White yam)	7.1×10^4	1.39×10^5	*Bacillus subtilis, Bacillus cereus, Pseudomonas sp, Rhizopus stolonifer, Flavobacterium sp, Aspergillus flavus, Penicillium oxalicum, Aspergillus niger, Enterobacter spp*
CONTROL	Water yam	1.6×10^1	2×10^1	*Bacillus cereus*
CONTROL	White yam	4.2×10^1	2.8×10^1	*Bacillus cereus and Bacillus subtilis*

Figure 1: Different processed yam products being sold at (a) Oja Oba market (b) Bodija market (c) crushed samples being blended (d) pulverized sample (e) peeled control samples.

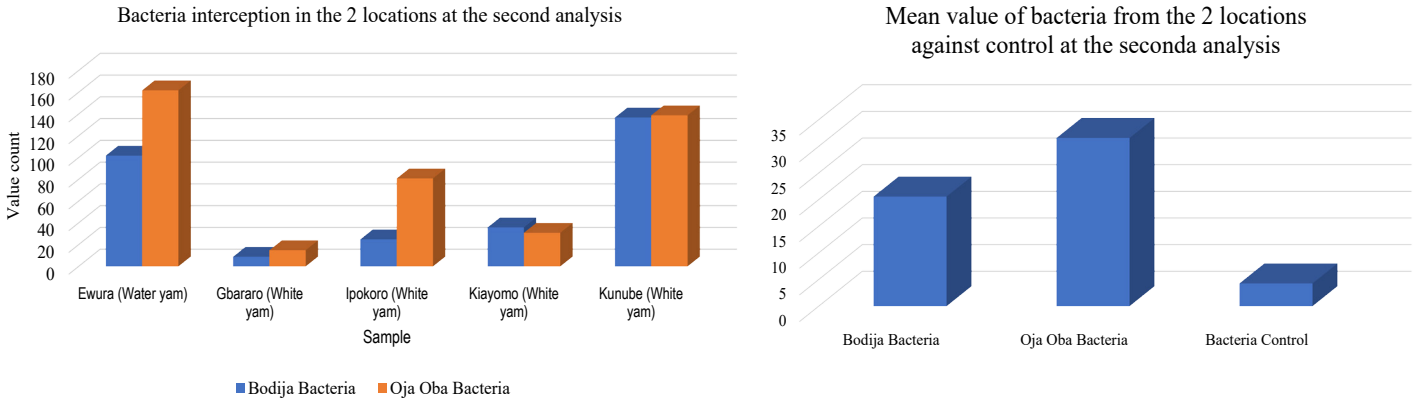

Figure 2: Showing mean value of bacteria isolated at first analysis of the Different Yam Samples.

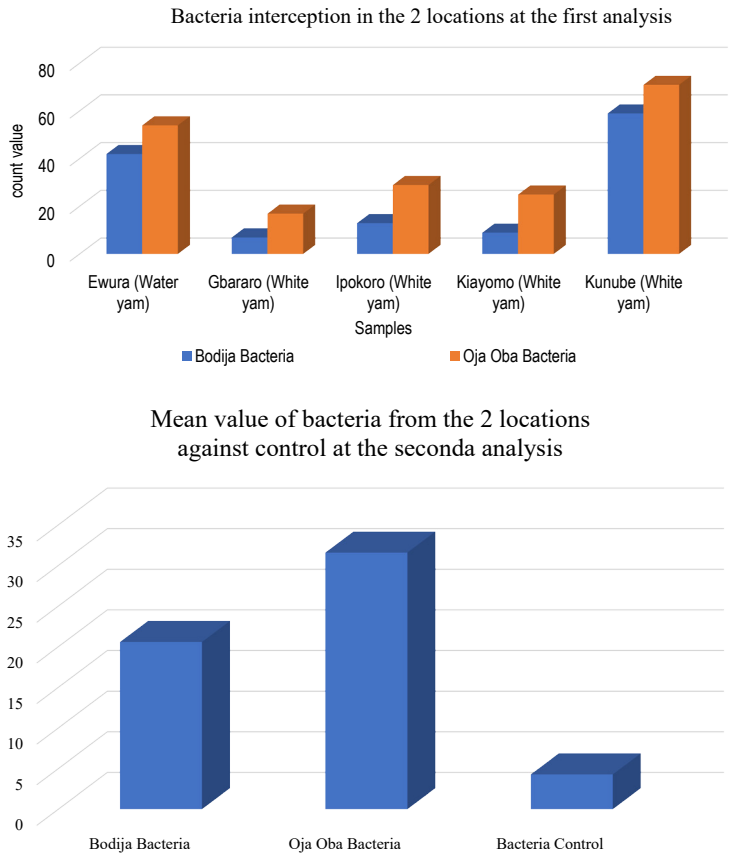

Figure 3: Showing Mean Value of Microbes Isolated at First Analysis of the Different.

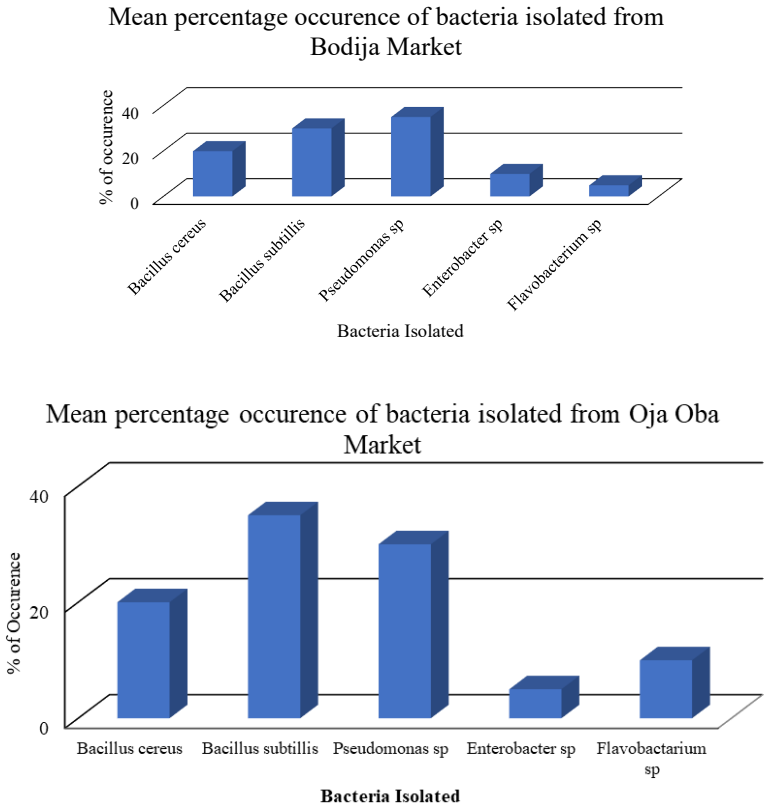

Figure 4: Showing percentage mean occurrence of bacteria isolated from both markets.

Table 3: Random biochemical test.

Random bacteria biochemical reactions test															
Samples	ID	Gram reaction	Aerobic	Anaerobic	Motility	Catalase	Oxidase	Urease hydrolysis	Citrase Hydrolysis	Starch hydrolysis	Nitrate production	Casein Hydrolysis	H_2S Production	Gelatin hydrolysis	Organism Name
BDJ Ewura	102	positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
BDJ Ewura	103	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
BDJ IP	104	negative	+	-	Non Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
BDJ IP	102	negative	-	+	Motile	+	-	+	+	-	-	-	-	-	*Enterobacter sp*
BDJ KIA	103	negative	+	-	Non Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
BDJ KIA	105	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
BDJ KIA	103	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Flavobacterium sp*
BDJ KIA	101	positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
BDJ KIA	102	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Flavobacterium sp*
BDJ GBA	101	positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
BDJ GBA	103	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
BDJ IP	101	Positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
BDJ KUN	103	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
BDJ KUN	104	negative	+	-	Non Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
BDJ KUN	106	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
Control water	101	positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
Control water	101	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
Control white (A)	101	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
Control white (B)	101	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB GBA	102	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
OJB GBA	103	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB Ewura	102	positive	+	-	Non Motile	+	-	+	+	+	+	+	-	-	*Bacillus cereus*
OJB Ewura	103	positive	-	+	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB Ewura	103	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
OJB IP	103	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
OJB IP	102	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
OJB IP	101	Negative	+	+	Motile	+	-	+	+	-	-	-	-	-	*Enterobacter sp*
OJB IP (A)	102	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB IP (B)	102	Positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB KIA	101	negative	+	+	Motile	+	-	+	+	-	-	-	-	-	*Enterobacter sp*
OJB KIA	105	positive	+	-	Non Motile	+	+	+	+	+	+	+	-	-	*Bacillus subtillis*
OJB KUN (A)	103	positive	+	-	Non Motile	+	+	+	+	-	-	-	-	-	*Bacillus subtillis*
OJB KUN (B)	103	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Flavobacterium sp*
OJB KUN	104	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*
OJB KUN	103	negative	+	-	Motile	+	-	+	+	-	-	-	-	-	*Enterobacter sp*
OJB KUN	105	negative	+	-	Motile	+	+	+	+	-	-	-	-	-	*Pseudomonas sp*

had been *Bacillus sp, Pseudomonas sp, Flavobacterium sp, Enterobacter sp, Rhizopus stolonifer, Aspergillus niger, Penicillium oxalicum, Mucor, Aspergillus flavus.* The *Flavobacterium spp.* and *Pseudomonas spp.* are rugged and opportunistic, gram-negative rods, non-spore-forming, strictly aerobic, motile by gliding, pigmented bacteria [24] while *Enterobacter spp.* is anaerobic. Table 3, showed random biochemical test of bacteria isolated. *Flavobacterium spp. Enterobacter spp.* and *Pseudomonas spp.* are distributed widely in nature being observed in water, saline solutions, utensils or even in cosmetics, prescription drugs and disinfectants, lots of herbal, synthetic foods and have been isolated from various habitats and are opportunistic or proper pathogens that purpose disorder in an extensive sort of organisms, which include plants, fish, and humans [25,26]. They can continue to be possible for lengthy intervals of time in lots of exclusive habitats and below very detrimental conditions. *Bacillus spp* was isolated due to their ability to endure various adverse environmental conditions and if not properly processed, it might lead to food poisoning [27]. Importantly, yam flour infection with psychotropic microorganisms is a specific subject as they're disbursed at temperatures permit the increase of those organisms. Psychotropic microorganisms are the ones capable of proliferate at or underneath 7°C, no matter their surest increase temperature. The study shows that the infection degree of *Flavobacterium spp., Enterobacter spp.* and *Pseudomonas spp.* turned into now no longer notably exclusive in numerous places of marketplace sites. Regardless of the repute and grades of the marketplace, they have the equal chance to introduce spoilage microorganism like *Pseudomonas spp.* and *Flavobacterium spp.* from yam to meals in the event that they forget accurate meals managing measures. Hence if commercially processed and advertised yam merchandise are infected, it can represent a method of fitness danger to human

if fed on without being well cooked [8]. Therefore, it is essential to cook yam products very well to reduce the contamination below harmful levels.

Conclusion

Personal hygiene is exceptionally endorsed in right dealing with of the yam tubers for the duration of the put-up processing. It is usually recommended that if industrial processors of dry yam tubers may also have a commercial drying system or dryer with a view to assist reduce the degree of microbial invasion via surroundings for the duration of growth, maturation and dealing with methods.

During the milling process, it's miles very vast that the processors of yam flour must recognize the significance of everyday cleansing in their milling machines and keep away from the gathering of flour spilled at the ground into the lot to be consumed. Appropriate storage situation and packaging material is likewise endorsed in storage and packaging of dry yam flour [28,29]. It's exceptionally crucial that the authority's organizations must check out each market and processing sites at everyday interval to look if there may be adherence to the laid down preferred running procedure. This will assist food regulators in Nigeria to apprehend the state of affairs of practices hired through the manufacturers and dealers of dry yam samples with a view to make sure that the quality practices are complied with and customers could have got entry to more secure yam flour. Sensitization packages and recognition of meals protection must additionally be executed so that it will assist them apprehend the viable fitness dangers related to such unhygienic practices mainly with inside the wake of the growing food poisoning instances suggested because of intake of yam flour meals.

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