International Journal of Scientific Engineering and Applied Science (IJSEAS) - Volume-1, Issue-7,October 2015
ISSN: 2395-3470
www.ijseas.com

MICROBIOLOGICAL QUALITY EVALUATION OF LOCALLY PREPARED SNACKS SOLD IN ABA METROPOLIS, ABIA STATE, NIGERIA.

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ABSTRACT

Microbial contamination of locally prepared snacks sold by street vendors in Aba Metropolis, Abia State, Nigeria has become a health concern as they are consumed by huge population. This study was undertaken to investigate the microbiological quality of locally prepared snacks like meat pies, fish pies, plantain chips and potato chips sold in Aba Metropolis, Abia State. A total of one hundred and twenty (120) samples were aseptically collected from four (4) different busy locations and analyzed by standard microbiological methods to determine the colony forming units per gram of samples. Microbial bioloads of different microbial groups for the four different busy locations studied showed, total aerobic bacterial counts ranges from (2.1±0.70) $x10^2$ CFU/g to (4.0±0.15) $x10^4$ CFU/g, coliform count ranges from (0.7±0.36) $x10^1$ CFU/g to (2.6±0.45) $x10^2$ CFU/g and fungal count ranges from (0.4±0.67) $x10^1$ CFU/g to (2.2±0.23) $x10^3$ CFU/g. Seven different bacterial and four fungal isolates were identified to include *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* species, *Pseudomonas* species, *Clostridium* species, *Enterococcus* species, *Klebsiella* species, and *Aspergillus* species, *Penicillium* species, *Rhizopus* species, *Mucor* species respectively. All the samples studied showed presence of coliform. The results indicated that the studied samples were heavily contaminated, indicating non-enforcement of regulatory acts and food safety procedures to hazard analysis critical control points (HACCP) and good manufacturing practices (GMP). Adherence to GMP and HACCP during production and vending activities coupled with routine trade samples analysis are necessary in maintaining low microbial thresholds, an improved food safety management practices and preventing risk of associated food borne illnesses.

Key words: Snacks**,** Food safety, GMP, HACCP, Microbial thresholds.

Introduction

In Nigeria today, most people depend on snacks for a significant portion of their nutritional requirements. This is common among young generations – "the youths" (singles and students) with our young ladies occupying the greatest proportion of this class. A snack is seen in western culture as a type of food not meant to be eaten as a main meal of the day like breakfast, lunch or dinner but rather to assuage a person's hunger between meals, providing a brief supply of energy for the body [14]. Snacks are ready-to-eat food, raw or cooked, hot or chilled but ready for immediate consumption at the point of sale without further treatment [20]. Ready-to eat foods (RTE) are foods that are consumed in the same state as they are sold which include dried meat, fish and cereal based ready-to-eat food and do not include nuts in the shell, whole raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer [13]; [10]; [11]). [15] had earlier described snacks as popular articles of diet because they are appetizing in appearance, convenient in form, nutritious in content and give pleasing fullness to the stomach when consumed. The preparation and sale of street foods is an age-old activity. It is almost universal in developing countries, and in the industrial world. This activity has reached new dimensions as a result of rapid urbanization. The street foods are being served quickly, also tasty and it is available at reasonable rates. It sometimes attracts all the age groups, especially the younger generations.

The safety and shelf life of the street foods depends upon the interaction of chemicals, physical and microbial factors [8]. Street foods displayed on open yards can easily be contaminated by dust, exhaust smoke, insects, hands of intending buyers and climatic elements. The street food industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries. The industry feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. Recently, food borne illnesses emanating from street foods are major health challenge. The traditional processing methods used in preparations, inappropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended foods. Consumers who depend on such food are more interested in its convenience and cheap/ affordable price with little or no attention to its safety, quality, and hygiene [17]; [16]; [2]. Street foods are frequently associated with diarrhoea diseases which occur due to improper use of

additives, the presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices “GMPs” and good hygiene practices “GHPs” [8].

Vendors are often poorly educated, unlicensed, untrained in food hygiene practices, and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne diseases [3]. Most of the foods are not well protected from flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to street foods. Potential health risks are associated with contamination of food by *Escherichia coli, Salmonella typhi, Pseudomonas* species, *Staphylococcus aureus* during preparations and post-preparation stages [3]; [12]. Generally, snacks are divided into continental and local. Continental snacks include sandwiches, kebabs, hotdogs, meat pies, salad, dough nuts, and other bakery products while local snacks include roasted corn, roasted plantain (‘‘booli’’), plantain chips (‘‘ikpekere’’), potato chips, fried maize paste (‘‘kokoro’’) and so on. However, some of these continental snacks like meat pie and fish pie have been locally imitated, adulterated and produced; hence they are easily vended locally because of their huge market acceptance.

Meat or fish pie is a savory pie that contains filled minced meat or fish and other savory ingredients. The filled minced meat or fish is usually made of beef or smoked ice fish. They are baked pastry, made of flour dough that covers or contains meat or fish fillings and vegetables. They are the most popular of all pastries. Generally, baked foods are perishable foods with a short shelf life and they need special care in handling.

However, meat or fish pies have the tendency to spoil quickly. This is because of its fillings, which are mainly minced meat or fish, potatoes and little quantity of vegetables. Due to the high nutrient available in the fillings of the meat or fish pies, it makes microorganisms to thrive easily in the products [6]. Meat or fish pies are preferred taken hot/ warm and in an attempt to maintain this warmness, high voltage bulbs are normally used, and there are possibility of contaminating the meat or fish pies with spores of thermophilic bacteria such as *Clostridium perfringens*, a gram positive aerobe that grows best when not in competition with other organisms and at minimum temperature of 45^0C, its growth and replication is encouraged. There have been several cases of adulteration, where meat or fish pies were filled with potatoes or other savory ingredients instead of minced meat/ fish and it has been possible because it is difficult to see the fillings inside the meat or fish pie dough prior to purchasing. Also, there have been reported cases of spoilt meat or fish pies fillings at point of

purchase. Plantain and potato chips are prepared by washing the fruits, peeling, slicing, salting and frying the sliced fruits in hot edible oil. Thereafter, the fried sliced-fruits are packaged in potable and transparent nylon bags, ready for sale. Therefore, this study is targeted to assess the microbiological quality of locally prepared snacks sold in Aba Metropolis, Abia State, Nigeria.

Materials and Methods

Study area

The study area is Aba Metropolis, Abia State, in the South-East Geopolitical zone of Nigeria. The Aba town which has been known for ages as a major commercial center in the Eastern Nigeria is of the Igbo tribe and inhabited by Ngwa people. They are predominantly traders in the popular Ariria market. They have rich cultural history. Aba town is about 49km away from its state capital city, Umuahia and about 52km away from Port Harcourt city, the capital of Rivers State. The geographical coordinates are 5.1167^0N, and 7.3667^0E. The area is of tropical climatic conditions with rain forest features. The soil type is silt-clay and the weather is typical of rain forest, with an average annual temperature ranging between 25 - 35°C as lowest and highest values, respectively.

Sources of sample

The samples for the study were purchased from four (4) different busy locations in Aba metropolis. These busy locations are motor parks with close proximity to regular markets and have high percentage of locally vended snacks been patronized by customers, general public and intended travelers. These locations are Aba main motor park, Brass junction motor park, Ariaria motor park, and Osisioma motor park.

Sample collection

A total of one hundred and twenty (120) samples of locally vended snacks (meat pies, fish pies, plantain chips and potato chips) randomly purchased from four different busy locations in Aba metropolis. Random samples of different locally vended snacks were aseptically collected with sterile ziploc bags. The samples collected were for both microbiological and physicochemical analysis. Samples were analyzed in the laboratory within thirty (30) minutes of collection for both microbiological and physicochemical analysis.

Microbiological analysis of samples

Ten fold serial dilutions of samples were done. Spread plate and streaking culturing techniques [5] were used to enumerate and isolate bacteria and fungi in the samples. One (1) gram of each food samples was aseptically blended and homogenized in 10 ml of sterile distilled water (10^{-1} dilution). Serial dilutions of the homogenates were made to 10^{-2} and 10^{-3} and each dilution was plated in replicate using Plate count agar for total heterotrophic bacteria count and isolation of bacterial isolates, M-Endo medium for coliform count and isolation of isolates, Sabaraud Dextrose agar (SDA) plus chloramphenicol for fungal count and isolation of isolates and Mannitol salt agar for Staphylococci isolation. Pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of [4]. The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique, and lactophenol staining. The schemes of [1], and [21] were used for the identification. The plates were incubated at 35 ± 2°C for 72 hours ± 2 hours and 24 hours ± 2 hours (total bacterial count and coliform count respectively) and 25 ± 2°C for 120 hours ± 2 hours (fungal count).

Physico-chemical studies

The physicochemical parameters measured include pH and temperature. They were determined using methods of [7].

Data analysis

Data obtained from this research work were analysed using ANOVA. Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 16.

Results

The mean total aerobic plate counts of snack samples from the four (4) different vending sites are shown in Table 1. It showed that meat and fish pies from Ariaria park and Main park had significant higher counts compared to other samples. Meat pies had the highest count (4.0±0.15) $x10^4$ CFU/g followed by fish pies (5.0±0.18) $x10^3$ CFU/g, potato chips (3.2±0.74) $x10^3$ CFU/g and plantain chips (2.9±0.63) $x10^3$ CFU/g in that order. It also revealed that samples from Ariaria park had

relatively higher counts compared to other vending sites. Ariaria park had the highest counts followed by Main park, Osisioma park and Brass park respectively.

Table 2 and 3 showed the mean coliform and fungal count s of snack samples from the four (4) different vending sites. Results shows that recorded counts had similar trend with that obtained in total aerobic bacteria count. Meat pies had the highest coliform/ fungal counts (2.6±0.45) $x10^2$ CFU/g/ (2.2±0.23) $x10^3$ CFU/g, followed by fish pies (2.2±0.62) $x10^2$ CFU/g/ (1.2±0.90) $x10^3$ CFU/g, potato chips (1.4±0.90) $x10^2$ CFU/g/ (1.2±0.08) $x10^2$ CFU/g and plantain chips (1.0±0.11) $x10^1$ CFU/g/ (0.7±0.55) $x10^2$ CFU/g. All microbiological parameters showed some pattern of trend except for physicochemical parameters (pH and temperature) that did not show any observable trending (Table 4). The values obtained between the various snacks samples, when compared were statistically significant ($p<0.05$).

Discussion

It was observed that appropriate hygienic conditions in terms of personal hygiene, good manufacturing and food safety practices were not followed by the producers and vendors/ hawkers in this investigation. The presence of these organisms can be linked to a number of factors such as improper handling and processing conditions by the producers, use of contaminated water during washing and processing, cross contamination from raw materials used during production and the use of dirty processing utensils like mixing bowls, knives and trays. However, most of the producers and hawkers are unaware of food regulations in Nigeria and have no training in food safety related matters, hence this gap needs to be bridged.

There are high microbial counts recorded in the results in Tables 1, 2 and 3. These high microbial counts experienced with samples of meat and fish pies in total aerobic bacterial counts, coliform counts and fungal counts are higher than that of potato and plantain chips. It could be recalled that meat and fish pies are baked while plantain and potato chips are fried. The high microbial loads in the meat and fish pies could be linked to the heavy nutritious inner fillings that are wrapped with the dough. The grounded meat and fish used in the fillings are perishable on their own, and contains high proportion of proteins that supports microbial growths and replications. These high microbial loads are within threshold limits, although considering microbial replication pattern which occurs per second for bacteria, some samples with very high counts can pose serious health threats in few days.

The presence of coliforms could be traced to poor sanitary practices of food handlers and is an indication of faecal contamination. The presence of *Escherichia coli and Enterococcus* species in the meat and fish pies is an indication of poor sanitary conditions in the management of animal intestines in the abattoir slaughter house as both organisms inhabits in the abdomen and is a further support to possibility of faecal contamination in the products. *Escherichia coli* has been identified and used as in indicative microorganism for faecal contamination in food safety industries, and its presence points to poor hygiene practices by food handlers. The presence of coliforms in plantain and potato chips are clear indication of poor hygiene practices by food handlers (both producers and hawkers). In meat and fish pies prevalence, *Staphylococcus aureus* was the highest, followed by *Pseudomonas* species and *Bacillus* species respectively, while in plantain and potato chips prevalence, *Staphylococcus aureus* was the highest, followed by Bacillus species and *Pseudomonas* species respectively. *Bacillus, Clostridium, Klebsiella* and *Pseudomonas* species are known to be environmental contaminants, considering rate of exposure of these locally made snacks by most vendors/hawkers, while *Staphylococcus aureus* is known to inhabit on human skin as an opportunistic microorganism. *Bacillus* and *Clostridium* species are known as spore formers and can be found in the air, water and soil and can withstand harsh weather conditions, hence can contaminate vended snacks as the vending activities takes place in busy-crowded environments. The presence of *Aspergillus, Penicillium, Mucor* and Rhizopus could be attributed to the surrounding environment and packaging materials. These results obtained in this study are in agreement with the works of [6] and [18].

A close observation on Table1-3 for microbial counts and Fig. 1-2 for microbial prevalence of different isolated species shows that meat pies had the highest microbial count and prevalence when compared with other samples, followed by fish pies, potato chips and plantain chips respectively. Table 4 shows that locally prepared snacks maintained an optimum holding temperatures (at par with room temperature) and pH for the survival of most bacterial species and very few fungal species. The low microbial counts observed with plantain and potato chips are attributed to the inhibitory activity of oil used in frying the snacks. However, frying oil maintains temperatures as high as 140^0C which can kill spore formers, and could function as a preservative/ inhibitor by creating non-conducive environment for microorganisms by sealing up the air pores and functioning as a heat trap. This leads to the low microbial counts and prevalence recorded in the fried snacks samples. There is statistical significance among different values obtained in the results ($p<0.05$).

Conclusion

The results of the study revealed high microbial contamination of the locally prepared snacks which could lead to food borne outbreaks. Therefore, it is advisable that an awareness campaign should be carried out to educate the major players in the snacks industry on the approach of food safety management, associated vending hazards and critical points to be monitored so as to create consumers confidence and safety of the consumed snacks.

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Table 1: Mean Total Aerobic Bacteria Count

Snacks samples	**Sample sources**			
	Main park	**Brass park**	**Ariaria park**	**Osisioma park**
Meat pies (CFU/g)	$(3.3\pm0.61^{b}) \times 10^{4}$	$(3.4\pm0.32^{d}) \times 10^{3}$	$(4.0\pm0.15^{a}) \times 10^{4}$	$(3.7\pm0.44^{c}) \times 10^{3}$
Fish pies (CFU/g)	$(4.5\pm0.47^{b}) \times 10^{3}$	$(2.1\pm0.70^{d}) \times 10^{2}$	$(5.0\pm0.18^{a}) \times 10^{3}$	$(2.5\pm0.38^{c}) \times 10^{2}$
Plantain chips (CFU/g)	$(2.2\pm0.19^{b}) \times 10^{3}$	$(2.2\pm0.50^{d}) \times 10^{2}$	$(2.9\pm0.63^{a}) \times 10^{3}$	$(2.4\pm0.19^{c}) \times 10^{2}$
Potato chips (CFU/g)	$(1.8\pm0.44^{b}) \times 10^{3}$	$(2.6\pm0.09^{d}) \times 10^{2}$	$(3.2\pm0.74^{a}) \times 10^{3}$	$(2.8\pm0.88^{c}) \times 10^{2}$

Within rows, values with the same letters are not significantly different.
Standards: Total aerobic bacteria count (TABC) = $\leq 10^{5}$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^{4}$/g
[19]; [9].

Table 2: Mean Coliform Count

Snacks samples	**Sample sources**			
	Main park	**Brass park**	**Ariaria park**	**Osisioma park**
Meat pies (CFU/g)	$(2.3\pm0.50^{b}) \times 10^{2}$	$(1.7\pm0.09^{d}) \times 10^{2}$	$(2.6\pm0.45^{a}) \times 10^{2}$	$(1.9\pm0.30^{c}) \times 10^{2}$
Fish pies (CFU/g)	$(1.8\pm0.19^{b}) \times 10^{2}$	$(1.7\pm0.88^{d}) \times 10^{1}$	$(2.2\pm0.62^{a}) \times 10^{2}$	$(1.8\pm0.09^{c}) \times 10^{1}$
Plantain chips (CFU/g)	$(0.9\pm0.10^{b}) \times 10^{1}$	$(0.5\pm0.33^{d}) \times 10^{1}$	$(1.0\pm0.11^{a}) \times 10^{1}$	$(0.7\pm0.36^{c}) \times 10^{1}$
Potato chips (CFU/g)	$(2.1\pm0.55^{b}) \times 10^{1}$	$(1.3\pm0.26^{d}) \times 10^{1}$	$(1.4\pm0.90^{a}) \times 10^{2}$	$(1.5\pm0.14^{c}) \times 10^{1}$

Within rows, values with the same letters are not significantly different.
Standards: Total aerobic bacteria count (TABC) = $\leq 10^{5}$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^{4}$/g
[19]; [9].

Table 3: Mean Fungal Count

Snacks samples	Sample sources			
	Main park	**Brass park**	**Ariaria park**	**Osisioma park**
Meat pies (CFU/g)	$(1.9\pm0.42^{b}) \times 10^{3}$	$(1.5\pm0.17^{d}) \times 10^{2}$	$(2.2\pm0.23^{a}) \times 10^{3}$	$(1.7\pm0.67^{c}) \times 10^{2}$
Fish pies (CFU/g)	$(1.5\pm0.53^{b}) \times 10^{2}$	$(1.3\pm0.34^{d}) \times 10^{2}$	$(1.2\pm0.90^{a}) \times 10^{3}$	$(1.5\pm0.11^{c}) \times 10^{2}$
Plantain chips (CFU/g)	$(1.0\pm0.07^{b}) \times 10^{1}$	$(0.4\pm0.67^{d}) \times 10^{1}$	$(0.7\pm0.55^{a}) \times 10^{2}$	$(0.6\pm0.80^{c}) \times 10^{1}$
Potato chips (CFU/g)	$(1.0\pm0.98^{b}) \times 10^{2}$	$(0.7\pm0.20^{d}) \times 10^{1}$	$(1.2\pm0.08^{a}) \times 10^{2}$	$(0.8\pm0.07^{c}) \times 10^{1}$

Within rows, values with the same letters are not significantly different.
Standards: Total aerobic bacteria count (TABC) = $\leq 10^{5}$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^{4}$/g
[19]; [9].

Table 4: Physicochemical Results

Snacks samples	Sample sources							
	Main park		**Brass park**		**Ariaria park**		**Osisioma park**	
Sample Parameters	**pH**	**Temp (^{0}C)**	**pH**	**Temp (^{0}C)**	**pH**	**Temp (^{0}C)**	**pH**	**Temp (^{0}C)**
Meat pies (CFU/g)	6.6 ± 0.92^{cd}	37 ± 0.17^{a}	6.7 ± 0.31^{bc}	37 ± 0.25^{a}	6.9 ± 0.18^{a}	37 ± 0.17^{a}	6.8 ± 0.65^{ab}	37 ± 0.09^{a}
Fish pies (CFU/g)	6.6 ± 0.14^{bc}	36 ± 0.20^{b}	6.5 ± 0.97^{cd}	37 ± 0.08^{a}	6.8 ± 0.71^{a}	36 ± 0.20^{b}	6.7 ± 0.08^{ab}	37 ± 0.36^{a}
Plantain chips (CFU/g)	6.6 ± 0.76^{a}	36 ± 0.64^{b}	6.4 ± 0.15^{bc}	36 ± 0.76^{b}	6.5 ± 0.26^{ab}	37 ± 0.64^{a}	6.4 ± 0.57^{bc}	36 ± 0.55^{b}
Potato chips (CFU/g)	6.4 ± 0.09^{a}	37 ± 0.53^{a}	6.2 ± 0.22^{bc}	36 ± 0.43^{b}	6.3 ± 0.05^{ab}	37 ± 0.53^{a}	6.2 ± 0.66^{bc}	37 ± 0.81^{a}

Within rows, values with the same letters are not significantly different.

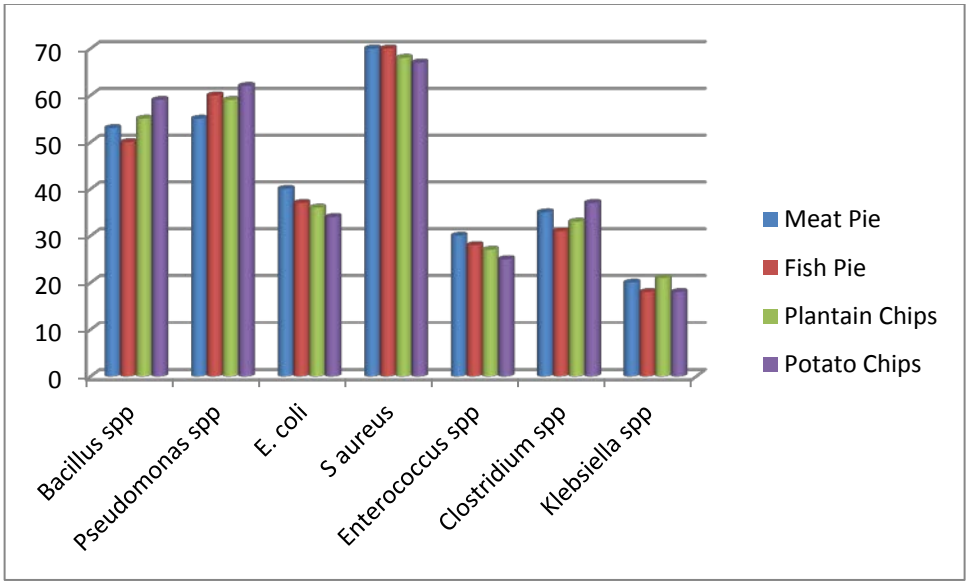

Fig. 1: Percentage prevalence of bacterial isolates from samples

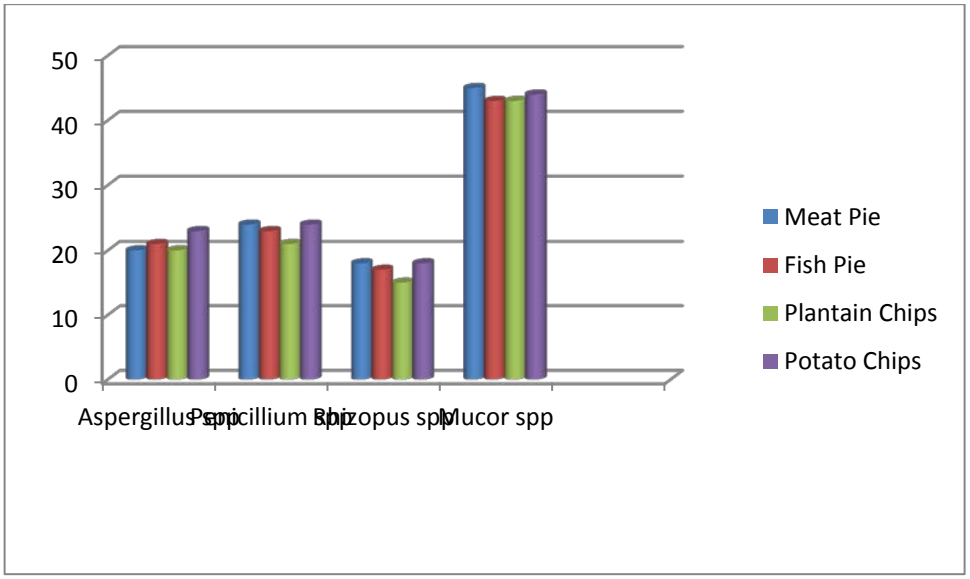

Fig. 2: Percentage prevalence of fungal isolates from samples