

Isolation and Identification of Moulds Associated with Four Selected Snacks Sold in Nnamdi Azikiwe University, Awka and its Environs

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ABSTRACT— *Food contamination is a major problem associated with food and snacks hawking in our society today. At a time when a lot of resources are being pumped into improving consumer health through food, it is ironic that more and more people are getting sick or dying from what they eat because of safety slips. This study was, therefore, carried out to determine the level of mould infestation associated with four selected snacks namely meat pie, buns, egg roll and doughnut. The snacks were purchased from vending sites in Amawbia, Aroma, Eke-Awka and Ifite all within Awka, Anambra State. Nnamdi Azikiwe University is within Ifite. Results obtained showed that seven moulds species were associated with the selected snacks. The mould species isolated were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium digitatum* and *Rhizopus sp.* Mean total fungal count from the four snacks was significantly higher in Meat pie (21.4 ± 2) and Egg roll (15.3 ± 3) than in Doughnut (6.2 ± 2) and Buns (5.9 ± 1.6). Also, Eke Awka had mean total fungal count of 18.03 ± 3 which was significantly higher than at any other location. The lowest mean fungal count of 8.75 ± 1.8 was recorded at Amawbia. There was variation in the pattern of occurrence of the moulds in the snacks with *P. digitatum* having the highest percentage occurrence in all the locations put together.*

Keywords--- Food, Contamination, Snacks, Infestation, Moulds

1. INTRODUCTION

In most parts of Nigeria, people depend on snacks for a significant portion of their nutritional requirements. 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 (James, 2005). 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 (Tsang, 2002). According to Gilbert *et al.* (2000), ready to eat foods are foods that are consumed in the same state as they are sold and do not include nuts in the shell, whole raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer. Miller and Ramsden (1955) 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.

Generally, snacks are divided into continental and local (Liyide, 2010). Continental snacks include sandwiches, kebabs, hotdogs, meat pie, salad, dough nuts, and other bakery products while local snacks include roasted corn, roasted plantain ("booli"), plantain chips ("ikpekere"), fried maize paste ("kokoro") and so on. These foods can serve as main meals or in-between meals for children and adults (Olumakaiye and Ajayi, 2008). Mensah *et al.*, (2002) reported that young children and adolescents do a good deal of snacking while parents supplement their babies' diet with snacks. Some snacks like potato chips, carry not only excess calories but also surplus fat and salt. While some snacks do not provide the best balance of nutrients, they may be fortified with various nutrients though some can cause weight gain in consumers (Foskett *et al.*, 2004). Snacks are usually sold along the road, in kiosks, eateries, and some are hawked from place to place, and on highways. The increase in the consumption of snacks has been associated with changes in social

patterns characterized by increased mobility, large numbers of itinerant workers and less family-centred activities (Odu and Akano, 2012). Also, convenience/modern life style, industrialization, economic downturn, quest for more wealth, materialism, and their associated lack of time to prepare proper meal and low purchasing power are some of the reasons advanced for the increased patronage for snacks (Nielsen, 2006). In addition, snack preparation and its sale provide employment and contribute to food security of the economy (Mensah *et al.*, 2001; Opeolu *et al.*, 2010).

The major problem associated with snacks is the frequent incidence of contamination. Due to the nature of these snacks and their methods of preparation involving extensive handling, they are usually prone to contamination from water, air, storage/distribution facilities, environment and human activities (food handlers and vendors) (James *et al.*, 2005; Oranusi *et al.*, 2011). Earlier studies on the assessment of microbial contamination have reported poor knowledge practiced in food handling (FAO, 1995). Furthermore, like many other processed foods, snacks are subjected to physical, chemical and microbiological spoilage by such organisms as *Escherichia coli*, *Salmonella*, *Clostridium*, *Staphylococci*, *Listeria*, *Bacillus* spp. and moulds of several genera like *Rhizopus*, *Aspergillus* and *Penicillium* (Smith *et al.*, 2004; Annan-Prah *et al.*, 2011; Odu and Akano, 2012). According to Coda *et al.* (2011), the major problem of long-term shelf life of snacks is contamination with fungi mostly of the genera *Eurotium*, *Aspergillus*, *Monilia*, *Mucor*, *Cladosporium*, *Fusarium* and *Rhizopus*. The implication of consuming contaminated snacks is the risk associated with ingestion of mycotoxins and other allergens produced by these contaminants.

The aim of this study is, therefore, to isolate and identify the various moulds associated with snacks sold in the selected areas. This study will provide information on the possible mycotoxins that contained in such contaminated snacks.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The laboratory aspect of this research was carried out at the Plant Pathology Laboratory of the National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State, Nigeria. Snack samples were collected from four locations namely Amawbia, Aroma, Eke-Awka and Ifite. These areas were chosen because they are popular places in Awka with high human and vehicular traffic and intensive food and snacks vending activities all through the week. The samples collected were five (5) each of fresh Buns, Meat pie, Egg roll and Doughnuts. The samples were purchased from different vendors from each location. All the samples were aseptically collected separately inside a sterile polyethylene bag totaling eighty (80) samples from all the four locations put together. The samples were then taken to the Plant Pathology Laboratory of NRCRI for subsequent studies.

2.2 Mould Isolation and Identification

To isolate moulds associated with each snack, one gram (1g) of each sample was homogenized in 9ml of sterile distilled water. This produces a homogenate used as a stock solution of each sample. Further serial dilutions of the resultant homogenates were made to the fourth diluent. Then, 1ml of each dilution was inoculated on sterile potato dextrose agar (PDA) in a Petri dish using the pour plate method (Fawole and Oso, 1988). Inoculated plates were sealed with paraffin wax and incubated at room temperature ($25 \pm 2^\circ\text{C}$) for 2-5 days with daily observation. The total number of fungal colonies per plate was counted using a colony counter. The number of individual mould colonies per plate was also recorded as well as the percentage frequency of each isolate.

$$\text{Isolate \% occurrence/location} = \frac{x \times 100}{n}$$

where x = total number of each organism in a location
 n = total number of all the organisms in a location

Total frequency of occurrence of organisms in all the locations was also calculated as previously described (Fawole and Oso, 1988) thus:

$$\text{Total \% occurrence} = \frac{X \times 100}{N}$$

where X = total number of organisms in a location
 N = total number of organisms in all the locations

2.3 Characterization and Identification of Fungal Isolates

On establishment of growth in the culture plates, the resulting growth was examined for the presence of discrete colonies from which transfers were made to fresh, sterile PDA plates. The plates were incubated as described earlier. The resulting culture plates were examined for uniformity of growth as an indication of purity. PDA bottle slants were subsequently prepared and stored in a refrigerator (4°C) for characterization and identification. Macroscopic and microscopic observations were carried out on the cultures. The physical characteristics of the mycelia such as the colour and structure were noted as well as the microscopic characteristics (Barnette and Hunter, 1987). Some morphological structures employed for identification include septation, presence/absence of sporangiophores, fruiting bodies and other special organs like the rhizoids. Statistical analyses were carried out using analysis of variance (ANOVA) to compare the occurrence of moulds among snacks and between locations.

3. RESULTS

A total of four genera comprising seven different species of moulds were isolated from all the snacks screened in all the locations covered by this study. The moulds are *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium oxysporum*, *F. solani*, *P. digitatum* and *Rhizopus* species. Degree of snack infestation with moulds differed among the different snacks and with location. Meat pie was the most heavily infested of the four snacks sampled while the least infested was buns (Table 1). The mean total fungal colony counts for the snacks are as follows: Meat pie (21.4 ± 2), Buns (5.9 ± 1.6), Doughnut (6.2 ± 2), and Eggroll (15.3 ± 3). Results for the total mean fungal colony count for the locations showed that Eke-Awka had the highest total mean fungal count (18.03 ± 3), followed by Ifite (11.7 ± 2), Aroma (10.33 ± 2) and Amawbia (8.75 ± 1.8) in this order (Table 2). Record of the frequency of occurrence of the individual moulds showed that the most frequent mould in the locations was *P. digitatum*. Some moulds did not occur in some snacks and some locations. Eke-Awka had the highest incidence of moulds in snacks under the conditions of this study while the least number of moulds was found at Amawbia. Statistical analyses showed that there were significant differences (at 5%) in the occurrence of moulds both between snacks and locations.

Table 1: Fungi Isolated from Four Selected Snacks Collected from Different Locations

Location	Sample	Moulds Isolated
Amawbia (A)	Meat pie	<i>A. flavus</i> , <i>F. solani</i>
	Buns	<i>F. solani</i>
	Eggroll	<i>P. digitatum</i>
	Doughnut	<i>A. niger</i> , <i>F. oxysporum</i>
Aroma (B)	Meat pie	<i>A. flavus</i> , <i>F. oxysporum</i>
	Buns	<i>P. digitatum</i> , <i>Rhizopus</i>
	Eggroll	<i>A. flavus</i> , <i>Rhizopus</i>
	Doughnut	<i>F. solani</i> , <i>F. oxysporum</i>
Ifite (C)	Meat pie	<i>A. flavus</i> , <i>A. fumigatus</i>
	Buns	<i>P. digitatum</i>
	Eggroll	<i>P. digitatum</i> , <i>Rhizopus</i>
	Doughnut	<i>P. digitatum</i> , <i>F. solani</i> ,
Eke Awka (D)	Meat pie	<i>P. digitatum</i> , <i>A. niger</i> , <i>A. fumigatus</i>
	Buns	<i>A. niger</i> , <i>Rhizopus</i>
	Eggroll	<i>F. solani</i> , <i>A. niger</i> , <i>Rhizopus</i>
	Doughnut	<i>P. digitatum</i>

Table 2: Mean Fungal Count of Snacks Analyzed in the Four Locations.

Locations	Mean Fungal count in Food Samples (10 ⁴)				Total(Mean)
	Meat Pie	Buns	Egg roll	Doughnut	
A	21.33±2	5.00±2.00	14.33±3.0	6.15±1.52	11.7±2
B	17.33±1.50	3.30±1.60	15.7±4.0	5.00±1.52	10.33±2
C	14.35±3	3.15±1.00	13.5±2.00	4.00±1.1	8.75±1.8
D	32.65±2	12.10±1.80	17.67±3.0	9.70±3.4	18.03±3
Total(Mean)	21.4±2	5.9±1.6	15.3±3	6.2±2	

4. DISCUSSION

The results of the present study revealed that different moulds were isolated from four snacks namely Buns, Meat pie, Doughnut and Eggroll collected from four locations (Amawbia, Aroma, Ifite and Eke Awka) in Awka. The isolated moulds were identified as food-borne. The moulds include *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium oxysporum*, *F. solani*, *P. digitatum* sp. and *Rhizopus* sp. Several researchers (Ogundare and Adetuyi 2003; Annan-Prah *et al.*, 2011 and Oranusi *et al.*, 2011) have reported similar moulds from different snacks. For instance, moulds from freshly baked bread included *Absidia corymbifera*, *Penicillium frequentans westing*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum* and their population increased with period of storage. The high incidence of moulds observed in this study could be associated with the very nutritious ingredients contained in the snacks. Chelea and Jideani, (1996) had reported that due to the high nutrient available in the fillings of meat pie, microorganisms thrive easily in it. Also Philips, (2003) reported that the major ingredients used in the preparation of Eggroll (eggs and hams) offer a rich nutrient medium for microbial growth.

Microbial Standards of the Public Health Laboratories of the Advisory Committee for the Food and Dairy Products classify certain foods as unsatisfactory. This has to do with the microbial loads of such foods implying that they are not good for consumption. Foods that have fungal load greater than the standard threshold of 10⁴ are considered unsafe (Philips, 2003). The snacks sampled in this study though have some moulds are not unsafe. The microbial load is quite below the threshold. However, there is need for more hygiene to be exercised while preparing these snacks so as to reduce the mould incidence. Permitted preservatives should be employed in the preparation of snacks. Potassium sorbate is often used to prevent yeast and mould growth in snacks. This preservative loses effectiveness if the pH is greater than 6.5. Another factor that exposes snacks to mould infestation is the handling involved as it concerns the hawking mode of distribution. Hawkers should be made to observe some certain level of hygiene in their distribution process. They should observe the “best before dates” on product labels for those products that have such labels. The UK Food Standards Agency (IFA) has recalled expired cakes and confectionery (Culliney, 2013).

The level of mould infestation was observed in this study to differ from one location to another. More moulds were isolated from snacks in Eke-Awka than from any other location. This must be related to the population and degree of filthiness in the different locations. Eke Awka is the biggest market in Awka, with immense high commercial activity and with people from neighbouring towns and villages bringing their produce for sale. This situation is responsible for the high human and vehicular traffic in this location with its attendant effect on the environment. The high infestation of snacks sold in this market can be traced to the dusty atmosphere usually prevalent in this location.

In conclusion, it is important that all major players in the snacks industry should be aware of the risks posed by snack contamination as it relates to consumption of mould toxins produced on contaminated snacks. Reports of loss of lives due to the activities of toxigenic moulds abound in literature and efforts should be made to reduce the risks. Therefore, there is need for systematic and universally applicable approach to food safety control.

5. REFERENCES

- Annan-Prah, A., Amewowor, D. H. A. K., Osei-Kofi, J., Amoono, S. E., Akorli, E. Saka, S. Y. and Ndadi, H. A., “Street Foods: Handling, hygiene and client expectations in a World Heritage Site Town, Cape Coast, Ghana”, *African Journal of Microbiology Research* 5(13): pp. 1629-1634, 2011.
- Barnette, H.L. and Hunter, B.B., *Illustrated Genera of Imperfect Fungi*; 4th edition Macmillian Publishing Company, New York, 1987.
- Chelea, M. and Jideani, I.A., “Incidence of Aflatoxin in some Ready to eat Foods sold in Market places in Bauchi Town”, *Food Journal* 17: pp. 51-65, 1996.
- Coda, R., Cassone, A., Rizzello, C. G., Nionelli, L., Cardinali, G., Gobbetti, M., “Antifungal activity of *Wickerhamomyces anomalus* and *Lactobacillus plantarum* during sour dough fermentation: identification of novel compounds and long-term effect during storage of wheat bread”, *In Appl Environ Microbiol.* 77(10):pp. 3484-3492, 2011.
- Culliney, K., Mold, expired dates and rat infestation prompt UK cake recall. www.bakeryandsnacks.com/Regulation-Safety/ 2013.
- FAO, *Street Foods Report of an FAO Technical Meeting on Street Foods*, Calcutta India, 1995.
- Fawole, M.O. and Oso, B.A., *Laboratory manual of Microbiology spectrum books Ltd Sunshine House Ibadan, Nigeria*, 1988.
- Foskett, T.A., Barrett-Connor E.L., Wingard, D.L., “Practical Cookery”, *Snacks, light meals, Savouries and Convenience Foods*.15:pp. 616-632, 2004.
- Gilbert, R.J., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C.D., Richards, J., Roberts, D. & Bolton, F.J., “Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale: A Working Group of the PHLS Advisory Committee for Food and Dairy Products”, *Communicable Disease and Public Health* 3: pp. 163-167, 2000.
- James, A.S., “The role of breakfast in the treatment of obesity: A randomized clinical trial”, *American Journal of Clinical Nutrition* 55:pp. 645-651, 2005.
- James, M.J., Martins, J.L. and David, A.G., *Modern Food Microbiology* 782pp, 2005.
- Liyide, B. O., *Frequency of Consumption of Local and Continental Snacks Amongst Secondary School Students in Abeokuta South Local Government Area of Ogun State*, 2010.
- Mensah, P., Armar-Klemensu, A., Hammond, S., Haruna, A. and Nyarko, R., “Bacterial contaminants in lettuce, tomatoes, beef and goat meat from metropolitan Accra”, *Ghana Med. Journal* 35:pp. 1-6, 2001.
- Mensah, P., Yeboah-Manu, D., Owusu-Darko, K. & Ablordey, A., *Street foods in Accra, Ghana: How safe are they?* Bull. World Health Organisation. Volume 8: 7pp, 2002.
- Miller, A. A. and Ramsden, F., “Contamination of meat pies by *salmonella* in relation to baking and handling procedures”, *Journal of Applied Bacteriology*, 18:pp. 565–580, 1955.
- Nielsen, A.C., “Ready to eat meals: Global highlights. A global consumer survey report. Safety Practice among Food Vendors in Ilorin”, *J. Med.* 5:pp. 120 – 124, 2006.
- Odu, N.N. and Akano, U.M., “The Microbiological Assessment of Ready-To-Eat-Food (Shawarma) In Port Harcourt City, Nigeria”, *Nature and Science* 10(8):pp. 1-8, 2012.

- Ogundare, A. O. and Adetuyi, F. C., “Studies on the microbial population of bread baked with wheat flour from south western Nigeria”, *Food, Agriculture & Environment 1(3&4)*: pp. 85-87, 2003.
- Olumakaiye, M. F. and Ajayi, O.A., Food Consumption Patterns of Nigerian Adolescents and effect on body weight, 2008.
- Opeolu, B.O., Adebayo, K., Okuneye, P.A. and Badru, F.A., “Physiochemical and Microbial Assessment of Roadside Food and Water Sample in Lagos Environs”, *J. Appl. Sci. Environ. 14*:pp. 29- 34, 2010.
- Oranusi, S., Omagbemi, F. and Eni, A.O., “Microbiological safety evaluation of snacks sold in fast food shops in Ota, Ogun State Nigeria”, *International journal of Agricultural and Food science 1(4)*:pp. 75-79, 2011.
- Phillips, M., “Analysis of Microbial Hazards related to time/temperature control of foods for safety”, *Comprehensive Review in Food Science and Food safety 2*:pp. 33-35, 2003.
- Smith, J. P., Daifas, D.P., El-khoury, W., Koukoutsis, J. and El-khoury, A., “Shelf life and safety concerns of bakery products – A review”, *In: Crit Rev Nutr. 44(1)*:pp. 19-55, 2004.
- Tsang, O., Guidelines for Ready-To-Eat Food. Road and Environmental Hygiene Department, Hong Kong, 2002.