



## EVALUATION OF MOLD SPECIES ON COOKED MAIZE FLOUR FOOD (TUWON MASARA) SOLD IN BIRNIN KEBBI METROPOLIS, NIGERIA

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### ABSTRACT

Microorganisms are associated with food in a variety way which become a global problem. Analyses of mold species on cooked food of maize (Tuwon Masara) were determined. Three (3) areas within Birnin Kebbi metropolis were sampled and selected for sample collections namely: Tsohuwar Tashi, Bayan Kara and Sabuwar Kasuwa. Ten (10) samples from each area were collected to make a total of Thirty (30) samples of Tuwon Masara. The samples were analyzed using pour plate method. Six (6) mold species were isolated and identified namely; *Aspergillus niger*, *A. flavus*, *Penicillium* spp, *Rhizopus stolonifer*, *A. fumigates* and *Mucor* spp. The results obtained showed that *A. niger* had the highest percentage of occurrences (34%) and least (6%) in occurrence was *Rhizopus stolonifer*. The result of mean fungal spore count showed that Tuwon Masara obtained from Bayan Kara had the highest ( $28 \times 10^{-3}$ sfu/g) fungal spore load while the lowest was recorded in Tsohuwar Tashi area with  $8.0 \times 10^{-3}$ sfu/gas compared to Sabuwar Kasuwa. The implication of this study could be that the handling, processing and sales of locally produced Tuwon Masara needs to be critically monitored to avoid contamination of this product by these isolates molds. It is also very necessary that local producers should be enlightened about good processing practices, handling, sanitation and the effect of proper preservation, process as this will ensure products free of pathogenic fungi.

**Keywords:** Mold species, load, and cooked maize flour food (Tuwon Masara)

### INTRODUCTION

Food is one of the basic substance consumed by living organisms on the earth. The production and processing of food provide job and income to many women in Birnin Kebbi and Nigeria at large. In fact food forms part of the culture of many Nigeria societies. Street foods are any cheapest ready-to-eat nutritive foods sold by food booths in an environment or places where people gathered (FAO, 2009).

Urban people mostly consumes and rely on street foods such as finger foods or fast foods as they are much cheaper in price than the foods sold in

restaurants. However, some people prepare to eat these booth foods sold on the street as they the tested better than the restaurant foods to them, while others could be as students, visitors, explorers, and individual differences as in types of food to be consume. Furthermore, street foods are topping popularity in Kebbi State due to increase in population.

The fundamental role of food booth in terms of providing daily nutritional diet that contains essential elements and are less expensive and provides street services to the communities but these are not being recognized more especially in

under developing countries as this provide economic and health benefits to the country (Dardano, 2003 and WHO, 2019). This ready-to-eat food provides micro and macronutrient that helps in building the human body and therefore improving greatly the health system sector and standard of living in both developing and developed countries (WHO, 2019). In addition, street-vended foods provides job opportunities and in come to women, self-employment and opportunity to develop business skills with low capital income, least expensive that provides most accessible means of obtaining good diets for many low income families (Dipeolu *et al.*, 2007; Loukieh *et al.*, 2001).

Tuwon Masara is one of the most common food consumed in Northern Nigeria more especially in the rural areas usually due to its availability and less as well as easy to cook. However, it has been traditionally consumed from generation to generation as the methods of preparations have been through since ages (Hadiza *et al.*, 2018). One of the recent challenge faced every nations is provision of healthy foods that are free from any contaminated microbes that improve the quality life (Paudyal *et al.*, 2017).

Therefore, mishandling of street foods, unhygienic environment and equipments, agitation of both vendors, buyers nearest to food and improper processing and preservation of foods by food booths increases the contamination of foods by these microbes resulted in food-borne diseases (Onyeneho and Hedberg, 2013; Schirone *et al.*, 2018). Nigeria is among the African countries that have cases of disease similar to borne-food diseases such as diarrheal basically in immunocompromised person or young children (Omemu and Omeike, 2010).

Several factors are used to ascertain food hygiene all over the world and these includes handling of food and foods items, equipment used during processing, preparation techniques and preserving practices (Ifediora *et al.*, 2006). Street foods posed major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending because of their diversity, mobility and temporary nature (Desousa, 2008).

Lack of expertise in the field of food and nutrition or mycologist, as well as lack of awareness by food booth and inadequate public

awareness of hazards posed by molds plays vital role in contamination of street foods which become global issue in recent years (Rane, 2011; Salamandane *et al.*, 2021). It is of concern that little or no effort has been made to study molds in Tuwon Masara at Birnin Kebbi metropolis, North-west Nigeria, upon all the role this local traditional food has in reduced hunger as well as boost the economy. Thus, this research work aimed to ascertain the molds proliferation of commercial ready-to-eat maize food (Tuwon Masara) in the study area.

## **MATERIALS AND METHODS**

### **Collection of samples**

Tuwon Masara samples were collected in sterile sample bags and labelled. Three (3) collection areas were selected for sample collection namely, Tsohuwar Tashi, Sabuwar Kasuwa and Bayan Kara area. Ten (10) plates containing samples of Tuwon Masara were collected in each sampling point which makes a total of thirty (30) samples. The entire collected samples were taken to the Botany laboratory of the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria for analysis.

### **Medium Preparation**

The appropriated volume of Potato Dextrose Agar (PDA) used was prepared according to manufacturer's instructions. Streptomycin was added involve 250 mg in order to inhibit the growth of bacteria (Cheesbrough, 2002; Keta *et al.*, 2020).

### **Serial Dilution and Inoculation**

Six test tube containing 9ml of distilled water were arranged in a test rack and one gram of local Tuwon Masara was inserted into the first test tube and was vigorously shaken. One ml from first test tube was transferred to second test tube continuously up to last test tube. One ml of diluted samples from test tube 4 and 5 were pipetted and dispensed into 20 petri-dish containing 20ml solidified PDA each. The plates were incubated at for 5-6 days with daily observed of mold growth. A new fresh plates containing 20ml solidified PDA were prepared accordingly and incubated at  $28\pm 30^{\circ}\text{C}$  for 5-7 days to obtained pure cultures.

### Identification of Mold Isolate

The pure isolates obtained were observed with naked eyes for the color appearance, spore form before microscopic examination. Clean grease free slide was used for the smear making. A small portion of the fungal mycelium was picked using a hot sterile inoculating needle and placed on the glass slide with a drop of Lactophenol blue stain put directly on the mycelium. Cover slide was used to cover the glass slide containing prepared mycelium and mounted on the microscope stage.

The viewing was done with the lower magnification (x4), then the higher magnification (x40) objective lens. The appearance, structure of the mycelium and types of fruiting body were observed and were compared with mycological Atlas as the criteria for the identification of the isolates (Fisher, 1988; Keta *et al.*, 2020).

### Statistical Analysis

Data was analyzed based on the percentage and frequency of occurrence of the isolated fungi as described by Keta *et al.* (2020) using formula as; Percentage of occurrences =

$$\frac{\text{No. of observ. in which species appeared} \times 100}{\text{Total no. of observations}}$$

### RESULTS

From the result obtained, six mold species were identified namely, *A. niger* (34%), *A. flavus* (17%), *Penicillium* spp (15%), *Rhizopus stolonifer* (6%), *A. fumigatus* (21%) and *Mucor* spp (7%). However, *A. niger* (34%) was recorded

with highest percentage as seen in (Table 1). Results of mean molds spore counts showed that Bayan Kara had the highest mold counts of  $28 \times 10^3$ cfu/g, followed by Sabuwar Kasuwa with  $21 \times 10^3$ cfu/g while the lowest ( $8 \times 10^3$ cfu/g) molds count was recorded in Tsohuwar Tashi (Table 2). Cultural and microscopic characteristics of molds isolates from Tuwon Masara are seen in (Table 3).

**Table 1:** The Occurrence of Mold Isolates from Maize Flour Meal (Tuwon Marasa) Sold in Birnin Kebbi Metropolis, Kebbi State, Nigeria

Fungi isolates	Number of occurrence	Percentage of occurrence %
<i>Mucor</i> spp	4	8
<i>A. flavus</i>	9	17
<i>Penicillium</i> spp	8	15
<i>A. niger</i>	18	34
<i>Rhizopus stolonifer</i>	3	6
<i>A. fumigates</i>	11	21
<b>Total</b>	<b>53</b>	<b>100</b>

**Table 2:** The Mean Mold Counts of Tuwon Masara Samples Sold in Birnin Kebbi Metropolis, Kebbi State, Nigeria

Fungal isolates	Collection Areas		
	Tsohuwar Tashi	Bayan Kara	Sabuwar Kasuwa
<i>Mucor</i> spp	$14 \times 10^{-3}$	$11 \times 10^{-3}$	-
<i>A. flavus</i>	$10 \times 10^{-3}$	$28 \times 10^3$	$21 \times 10^{-3}$
<i>Penicillium</i> spp	$12 \times 10^{-3}$	$13 \times 10^3$	$10 \times 10^{-3}$
<i>A. niger</i>	$20 \times 10^{-3}$	$25 \times 10^{-3}$	$18 \times 10^{-3}$
<i>Rhizopus stolonifer</i>	$8.0 \times 10^{-3}$	$17 \times 10^{-3}$	-
<i>A. fumigates</i>	$18 \times 10^{-3}$	$14 \times 10^{-3}$	$17 \times 10^{-3}$

**Table 3:** Morphological Characteristics of Molds Isolates from Tuwon Masara

Mold Isolates	Macroscopic	Microscopy
<i>Aspergillus niger</i>	Colonies were circular, opaque, smooth and flat with small black spots on short white hyphae.	Septate hyphae, branched conidiophore with secondary division. The conidiophore is enlarged at the tip forming rounding vesicle-like chains.
<i>Mucor</i> spp	Grows quickly and cover agar surface with white fluff that later turns grey, reverse side is white.	Hyphae practically non-septate, sporangiophores are long, often branched and bear terminal spore filled sporangia.
<i>A. fumigatus</i>	Creamy yellow filamentous colonies	Large/globose conidiophore, loose column with biserial hypha
<i>Penicillium</i> spp.	The colonies of are rapid growing, flat, filamentous and velvety, woolly, or cottony texture.	Chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous cell called a phialide.
<i>A. flavus</i>	Greenish yellow mycelium	Conidiophores septate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia.
<i>Rhizopus stolonifer</i>	Colonies are dark brown and the reverse side grew terminally	Hyphae were non septate with erect, simple branched sporangio-phores bearing rhizoids and sporangia

## DISCUSSION

The problems caused in contamination of food by these microbes cannot be over emphasized worldwide. The mold species isolated from Tuwon Masara in this research were namely, *Mucor* spp, *A. niger*, *A. flavus*, *Penicillium* spp, *Rhizopus stolonifer* and *A. fumigates*. Similar species were documented on handmade foods in some part of the world (Falola *et al.*, 2011; Oranusi and Braide, 2012).

The presence of these microbes could be probably due to several abiotic factors, and other activities that take place before reached the final stage of local Tuwon Masara and use of contaminated items. In developing countries, most of the food booth use contaminated water in preparation of handmade food which resulting in increase of ready-to-eat foods spoilage

(Salamandane *et al.*, 2021). The spore of some mold species are found in an environment as they natural habitant (Apinis, 2003).

However, occurrences of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* are probably due to the open environment where this food prepared and soon. This collaborated with the findings of Chelea and Jideani, (1996) and Oranusi *et al.*, (2011). The presence of molds in these food samples received much attention worldwide due to the health implication and toxigenic metabolites they produce on food and food products which are carcinogenic or mutagenic to man and his animals (Makun *et al.*, 2009).

Ekrakene and Igeleke (2007) stated that *Rhizopus* added significantly to food spoilage and food infection through the production of toxins.

A total of six (6) molds species were isolated and documented from ready to eat maize food (Tuwon Masara) in Birnin Kebbi metropolis. According to Keta *et al.* (2019), contamination of traditional street food in Birnin Kebbi are caused by microbes due to poor hygiene and sanitation.

The isolation of *Mucor* spp and *A. spp* corroborate the findings of Taulo *et al.* (2008); Oranusi *et al.* (2013); in which these organisms were implicated in ready-to-eat foods contamination. Out of the three location used in this study, Bayan Kara had the highest mean spore counter ( $28 \times 10^3$ ). This were higher than the one report on street ready-to-eat food in Kenya and Nigeria (Gitahi *et al.*, 2012; Aluko *et al.*, 2014). Unhygienic conditions increases count values of microorganisms on food and food products. Provision of healthy environments for food booth sellers, excessive heat during cooking and good storage facilities are the possible key factors that should be encouraged by government and stakeholders in preventing the spoilage of ready-to-eat food (Riener *et al.*, 2010).

The handmade foods and environment are contaminated due to negligence of food booth and street food buyers. This finding conforms to the studies by Christison *et al.* (2008). The Tuwon Masara is sold in the open usually by the roadside, in the middle of public places, markets and lorry stations. Openly displayed items like plates cannot be spared the presence of germs (Barro *et al.*, 2006). During the cough or bargaining for food or money, there is a passage of saliva onto food which increase the load of microbial substances. This act easily transfer whatever microbes present on the money to the food and the unsuspecting buyer would be the worst affected.

## CONCLUSION

Molds causes spoilage of food and inflammation diseases thereby putting the live of consumers at risk. Therefore, it can be concluded that, *Aspergillus niger*, *A. flavus*, *Penicillium* spp, *Rhizopus stolonifer*, *A. fumigatus* and *Mucor* spp were found associated with Tuwon Masara in the present study. These fungi isolated were capable of endangering human health more especially *Aspergillus* species due to their metabolites produced on foods and foods produces.

However, further studies in this direction are required. Therefore, handling, processing and sales of locally produced Tuwon Masara needs to be critically monitored to avoid proliferation of this product by these isolates molds.

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