



Analysis of Mold species Associated with the Granulated Maize Dumpling (*Dambu Masara*) Sold in Birnin Kebbi Metropolis

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ABSTRACT

Dambu Masara has been consumed in different parts of the world especially North western Nigeria. Its use is however limited to spoilage by microorganisms. This study was carried out to isolate and identify fungi species responsible for the spoilage of *Dambu Masara* in Birnin Kebbi. Two areas were selected for samples collection namely; Badariya and Go slow area all in Birnin Kebbi metropolis. Forty (40) samples of *Dambu Masara* were obtained in each sample site. A total of eighty (80) samples of *dambu masara* were collected all in all and analyzed. Using culture growth medium (Sabroud potato dextrose agar) and poured plate method fungi were analyzed. A total of five (5) fungal species were isolated and identified from the both samples as; *Aspergillus niger*, *Penicillium* spp., *Rhizopus stolonifer*, *Aspergillus fumigatus* and *Mucor* spp. The highest percentage of occurrences were obtained in *A. niger* (58.5%) while lowest were recorded *A. fumigatus* (2.1%). Base on locations, Go slow has the highest mold load of 83% while Badariya had the lowest 60%. Moulds are known to cause spoilage of *Dambu* products for the results obtained. Therefore when they are available in significant number, they lead to huge economic loss in addition to the pathogenic species causing health dangers. The implication of this study is that the process and quality of locally produced *Dambu* needs to be critically monitored to avoid transmission of infection to consumers.

Keywords: Mold species, Granulated Maize, Dumpling, (*Dambu Masara*)

INTRODUCTION

Street-vended food provide a major source of income for a vast number of persons, particularly women; a chance for self-employment and the opportunity to develop business skills with low capital investment; least expensive and most accessible means of obtaining a nutritionally balanced meal outside the home for many low income people (WHO, 2002; Dipeolu *et al.*, 2007). Cereal grains are the major source of food for most humans and domesticated animals in the world. There are many traditional foods of Northern Nigerian origin produced from cereal grains. These are *inter alia* (among others) *tuwo*, *kunu*, *fura*, *masa*, *burakosko*, *akamu*, *ndaleyi*, *danwake*, *gumba*, *sinasin*, *burukutu*, *dekkere*, *mardam*, *dakuwa*, *nakiya*, *bulum*, *pate pate*, *mpursa*, *kudon kaza*, *pito*, *nyiya*, *lamba* and *dambu* (Nkama *et al.*, 1998). The word *Dambu* refers to a cereal based granulated dumpling made from cereal flour, small quantity of onions, leaves and some herbs. *Dambu* is produced mainly from moistened millet grain flour blended with spices and steamed for thirty minutes. There are various types of *dambu* depending on the types of raw materials and methods of production. The various types include *dambun nama*, produced from either beef, mutton or poultry (Obodo, 2002; Balogun, 2008), *dambun kififi*, prepared from flesh of fish Goni (2002) and those prepared from cereal grains (Nkama *et al.*, 1998; Agu *et al.*, 2007; Agu *et al.*, 2008a). *Dambun nama* and *dambun kififi* are described as boiled, spiced, shredded, stirred and fried meat

and fish products, respectively. On the other hand, *dambu* produced from cereal grains is a granulated dumpling generally produced from moistened millet, sorghum, maize and fino millet flours or any suitable cereal flour blended with spices and steamed for about 30 minutes (Agu *et al.*, 2003a). Source of materials: the materials used include millet resembling moistened couscous. It is popular mid day meal (Agu *et al.*, 2007). *Dambu* is produced at home both for family and commercial consumption. Most *dambu* producers use the traditional method involving wooden mortar and pestle to dehull and mill the cereals grains. The traditional pounding process is a tedious task, which limits the use of cereal grains in most countries of Africa (Jideani *et al.*, 2001). The reports indicated that the ingredients used apart from cereals for the preparation of cereal based *dambu*, are cloves, ginger and black pepper. The addition of *M. oleifera* and *A. hypogaea* L. in the production of cereal based *dambu* are very important to human nutrition (Foidl *et al.*, 2001; Ogunsina *et al.*, 2010; Anjorin *et al.*, 2010). Moreover, because the cereal flour spoils quickly and cannot be kept for later use, it has to be milled perhaps on daily basis each time *Dambu* is needed. Furthermore, *dambu* like *fura* has a limited shelf life of 2 days at tropical ambient temperature (25-30°C) and also due to lack of packaging for this product (Jideani *et al.*, 2001). Increased moisture content on *Dambu* promotes the growth of microfungi, some of which are toxin producing like *Penicillium*, *Aspergillus* and *Fusarium*. Therefore, continued

consumption of these contaminated *Dambu* poses a health threat to the individuals. The mycotoxins produced are capable of causing acute and chronic effects in man (Turner, 2009). They cause hepatotoxic, nephrotoxic, neurotoxic, teratogenic, immunotoxic and mutagenic effects (Makun *et al.*, 2010) sometimes leading to death in man and other animals. Therefore the aimed of this presence study was to isolates and identify micro-fungi associated with *Dambu masara* sold in Birnin Kebbi metropolis

MATERIALS AND METHODS

Study Area

The study was conducted in Birnin Kebbi Local Government Area, Kebbi State. Birnin Kebbi is a city located in north Nigeria. It's the capital city of Kebbi State and headquarters of the Gwandu emirates. As of 2007 the city has an estimated population of 125,594. Birnin Kebbi are mostly Hausa and Fulani with other tribe. Birnin Kebbi is situated on the Sokoto river and is connected by road to Argungu (45km northeast), Jega (35km southeast) and Bunza (45km southwest). The annual temperature varies from 21°C to 38 °C with different soil type ranges from heavy clay in the fadama areas to loamy, sandy loam and sandy soils.

Sample Collection

Survey was conducted in major Dambu sellers for the samples collection *Dambu* Masara, two different areas was selected for samples collection namely; badariya and go slow. These sample were collected using stratify sampling methods. Each sample was collected in a sterile polythene bag separately, labelled and brought to Microbiology Laboratory of Kebbi State University of Science and Technology, Aliero.

Preparation of Sabouround Dextrose Agar (SDA) Medium

Sabouround Dextrose Agar (SDA) medium was prepared according to the manufacturer's instructions. Sixty (60g) of SDA was dissolved in 100ml distilled water. The water was heated to boiling point to dissolve the medium completely. The medium was then sterilize by autoclave at of 121°C for 15 minutes and mixed well before pouring into sterilized Petri dishes (Cheesbrough, 2002).

Sample Processing

One gram (1g) of each sample was aseptically weighed and homogenized using sterile mortar and pestle for one minute, the serial dilution was conducted and 1 gram of each homogenized sample was placed in a sterile conical flask containing 9 ml of sterile distilled water and mixed vigorously in order to obtain homogenized sample solution, 1-5 test tubes were filled with 9mls of sterile distilled water

and placed in the test tube pack at vertical position, 1ml from each homogenized sample solution placed into different conical flask was transferred into a first test tube containing water by using a sterile pipette and was capped and shaken vigorously and 1ml from second test tube was collected and transferred into a second test tube by using a sterile pipette and it was serially diluted to the last test tube (10^{-7}) (Oyeleke and Manga, 2008).

Isolation of fungi

A portion (0.1g) was pipetted from 10^{-4} and 10^{-5} dilution factor of each sample which were then aseptically inoculated on the solidified plates of SDA media respectively. The plates were incubated at 37°C for one week before colonies of fungi were counted and reported as colony forming units/g (CFU/g).

Sub-culture

Due to the appearance of different colonies, each colony was sub cultured into a new plate and incubated at 29±2°C for 5 days.

Identification of Fungi

The identification was conducted according to the method adopted by Barnett and Hunter (1987). Slides of pure cultures of the samples isolated were prepared for microscopic observation and identification. The culture and morphological characteristics of the isolates were observed and noted as the criteria that were used for identification of the fungi species described by Barnett and Hunter (1987). Detailed morphological characteristics of the fungi such as hyphae (septation), reproductive structure (sporangia/conidia) in chain or single; the type of spore were observed and recorded.

Descriptive statistics was done based on percentage and occurrence of isolates. Percentage frequencies of isolation (PFI) of all the fungi were calculated by the formular:

$$PFI = \frac{\text{No. of times a fungus is encountered}}{\text{Total no. of times all fungi were encountered}} \times 100$$

RESULTS

The results obtained showed that *Aspergillus niger* had the highest percentage of occurrence 29.5% and lowest was obtained in *A. fumigatus* 8.9% while *Penicillium* spp (23.3), *Rhizopus stolonifer* (5.8%) and *Mucor* sp (17.7) as presented in (Table 1). The location distribution of molds isolates showed that Go slow area had (83%) as the highest and Badariya was obtained with (60%) as the lowest as seen in (Table 2) respectively. However, morphology and Identification of mold isolates from both the both areas was shown in Table 3.

Table 1: Frequency and percentage of mold isolated from Maize dumpling (*Dambu masara*)

| Mold isolates | Frequency | Percentage (%) |
|----------------------------|-----------|----------------|
| <i>Aspergillus niger</i> | 10 | 29.5 |
| <i>Penicillium</i> sp | 08 | 23.5 |
| <i>Rhizopus stolonifer</i> | 02 | 5.8 |
| <i>Aspergillus flavus</i> | 05 | 14.7 |
| <i>Mucor</i> sp | 06 | 17.6 |
| <i>A. fumigatus</i> | 03 | 8.9 |
| Total | 34 | 100 |

Table 2: Location Distribution of Molds isolates

| Location | Mold isolates | Distribution (%) |
|-----------------|--|------------------|
| Badariya | <i>Aspergillus niger, Penicillium</i> sp, <i>Rhizopus stolonifer</i> | 60 |
| Go slow | <i>Penicillium</i> sp, <i>Aspergillus niger, A. fumigatus, Mucor</i> sp, <i>Aspergillus flavus</i> | 83 |

Table 3: Morphology and Identification of mold isolates from *Dambu masara* in both areas

| Sample | Macroscopic Examination | Microscopic Examination | Mold Isolated |
|----------|---|---|------------------------------|
| A | White in color at first and black. The reverse side was pale yellow. | Hyphae were septate, simple and walled. Conidiophores bearing heads containing conidial were seen | <i>Aspergillus niger</i> |
| B | Colonies were Grew | Sporangia were black and hard zygo-spores. Sporangiosphoreres were Long and branched hyphae were not Septate | <i>Mucor</i> species |
| C | Colonies were dark brown and the reverse side was grew terminally | Hyphae were non septate with erect, simple branched sporangioph- ores bearing rhizoids and sporangia | <i>Rhizopus stolonifer</i> |
| D | Greenish yellow mycelium | Conidiophores aseptate, unbranched with swollen apex. Conidiophores bear vesicles that produce chains of conidia | <i>Aspergillus flavus</i> |
| E | Dark green colony The culture was green diamond in colour and sparkling as it matured | The conidia are globe-like and resemble glass beads under the microscope. The hyphae appeared branching and septate conidiophores were erect, septate and faint | <i>Penicillium</i> sp. |
| F | Creamy yellow filamentous colonies | Large/globose conidiphore, loose columnna with biseriated hypha | <i>Aspergillus fumigatus</i> |

DISCUSSION

Food has always played an extra-ordinarily vital role in the rise and growth or the fall and decline of a nation because of its effect on the health of the population. Despite the economic and nutritional benefits of street foods, the consumption of these roadside foods has been suggested to potentially increase the risk of food borne diseases which cause considerable morbidity and mortality as street foods are readily contaminated from different sources (Tambekar *et al.*, 2008). The study had determined the various fungal pathogens associated with *dambu* maize as well as their grouping. A total of 5 isolates were recovered and document from the local prepared *Dambu masara* in Birnin Kebbi for the first time. This study has therefore shown that there are a number of fungal pathogens such as *R. stolonifer*, *A. niger*, *Penicillium* spp, *Mucor* spp, and *A. fumigatus*. The results of this study were in agreement with Bankole and Mabekoje (2004), which is in Nigeria various

grain mold fungi including *Fusarium*, *Penicillium*, *Aspergillus* and *Nigropora* spp. have been detected on maize samples. However, the absent of *Nigropora* spp in this present study could be due to the differences environment.

Ready to eat food (*Dambu masara*) and food preparation surfaces may be reservoirs for microbial contamination. This finding conforms to the studies by Mankee *et al.* (2005); Ghosh *et al.* (2007) and Christison *et al.* (2008). Pepper sauce may be contaminated from bowls in which they were served and more because the bowls were uncovered. The microbial quality of food is essential to ensure a safe product for the consumer but preventing contact with microorganisms is nearly impossible as food from food vendors are exposed to a wide range of microbes. The carry-over of potential pathogens from food vendors is also influenced by many environmental factors

including the microbial load present in the water used for cooking, type of food and a host of other single or interacting factors (Johnston *et al.*, 2006). Also, the mishandling and disregard of hygienic measures on the part of the food vendors again may enable microbes to contaminate food (WHO, 2009).

The vendors prepared the food at home on the average and conveyed to the vending point under unattractive conditions by means of public transport. From the place where the food is prepared, to the conveyance of the food to the sales point, there is the possibility of pathogenic microorganism getting into the food. The food is sold in the open usually by the roadside, in the middle of public places, markets and lorry stations. Openly displayed items like fried fish cannot be spared the presence of germs. It is disturbing to see clients standing right to bargain for such foods. During bargaining, there is a passage of saliva onto food which brings onto the food a load of microbial substances. The findings of the present study agreed with previous studies Mensah *et al.* (2002) and Barro *et al.* (2006) which indicated that the traditional processing methods that are used in preparation, in appropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food. Microbial contamination of food poses a serious threat to public health. The origin of microbial contaminant may either be environmental, natural or technological. To control the environmental or technological sources of microbial contamination, hygiene and cleanliness of food are two of the most important factors to consider (Riener *et al.*, 2010).

The presence of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus* could be attributed to the surrounding environment and packaging materials of Dambu masara. These results obtained in this study are in agreement with the works of (Goni, 2002; Makun *et al.*, 2010). The presence of *Mucor* sp., *Penicillium* sp., *A. niger*, *A. flavus*, *Fusarium* sp. and *R. stolonifer* in the food sample is not surprising as they disperse in the form of spores which is abundant in the environment and can be introduced through dust and soil (Apinis, 2003). The occurrence of *Mucor* spp and *Aspergillus* spp could be due to the fact that they are spore formers and their heat resistant spores may have survived processing while vegetative cells were eliminated. According to Bibeki (2001), contamination of food items by specific species of microorganisms is largely due to the presence of these organisms and their entrance into food or beverage as a result of poor hygiene and sanitation. The isolation of *Mucor* spp and *Aspergillus* spp corroborate the findings of Oranusi and Braide (2012); Taalo *et al.* (2008) in which these organisms were implicated in ready-to-eat foods.

CONCLUSIONS

Moulds are known to cause spoilage of *Dambu* products for the results obtained. Therefore when they are available in significant number, they lead to huge economic loss in addition to the pathogenic species causing health dangers. Raises concern over public health risks that may be associated with the consumption of *Dambu* may arise if

public enlighten, proper storage, good hygiene/environment and quality process equipment it not provides to reduce these microbes.

Microbial contamination by fungi may be considered as an important warning signal for human consumption of *Dambu masara*. Therefore, vendors should adapt better method of cooking, processing and preservation of this food. More so, it is important that mycological examination of *Dambu masara* be carried out, especially by environmental/ public health workers and regulatory bodies like Nigerian National Food and Drug Administration and Control (NAFDAC) at regular intervals and the health implications and consequences of eaten spoiled or contaminated *Dambu masara* should be made aware to the consumers as well.

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