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Original Research Article

Isolation And Identification of Fungi from Packed and Unpacked Milk, Corn Flour and Soya Bean

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Abstract

Fungi spoiling organisms are silently invading, acidifying, fermenting, discoloring, and disintegrating microbes that render food products unpalatable and unsafe for human consumption. This study aimed at isolation and identification of fungi from packed and unpacked milk, corn flour and soya bean. These samples were randomly purchased from vendors in Ekeonuwa market, Owerri and were analyzed using standard microbiological techniques. The findings of this study showed the presence of fungi contamination in these food products except for packed milk which had no fungi growth. The packed corn flour had a total fungi count of 3.1 x 10^4 Cfu/g, while soya bean flour had a total fungi count of 2.9 x 10^4 Cfu/g. However, the unpacked milk had a total fungus count of 3.2 x 10^5 cfu/g, corn flour had 3.7 x 10^5 cfu/g and soya bean had 3.0 x 10^5 cfu/g. Four genera of fungi were identified which are; Aspergillus, Rhizopus, Mucor and Penicillium. These fungi had various percentage occurrences. Aspergillus spp. was found to have the highest percentage occurrence of 35.7%, Penicillium spp. had 28.6%, Rhizopus spp. had 21.4% while Fusarium spp. had the least percentage of 14.3%. The recovery of these fungi from this research shows that there is fear of consumption of mycotoxins because of their serious health implication, as they can be highly toxic and carcinogenic, thus rendering the food products unfit for human and animal consumption.

Keywords: *isolation, identification, fungi, packed and unpacked milk, corn flour, soya bean.*

INTRODUCTION

Food products are a rich nutrient source that can attract both bacterial and fungal colonizers [1]. As such, the food product can be regarded as an ecological resource. Colonization with a number of food-borne microorganisms is beneficial with respect to nutritional value and prolonged storage of the food product, which is known as food fermentation in other case. After successful colonization of the product, its nutritional properties are altered [2]. When the nutritional value, structure, and taste of the product are negatively influenced, this colonization is called food spoilage. It can be accompanied by the production of toxic secondary metabolites which may result in serious medical problems [3]. These two aspects of food colonization are two sides of the same coin. Food spoilage is a major threat for our food stock and is responsible for enormous losses [4]

Fungi are the main degraders of the sturdy plant cell wall components that otherwise would accumulate within the ecosystems of the world. Prior to spoilage, the fungi can be present on or inside of the crop in low numbers, or as survival structures. Spoilage fungi can also be introduced to an empty habitat if the food is previously treated by pasteurization treatments. Food products include two main groups, which are living crops and processed food [5].

Colonization of food products is hence very diverse. The relationship between the living crop and fungi can be illustrated. Then the association of fungi with different types of processed food is described. Preservation techniques make the food product a difficult environment to colonize, although it is also a rich medium. Only fungi that can survive certain adverse conditions including high osmolarity and heat can successfully spoil processed food [6]. The overviews

on the taxonomic description and specificity of food spoilage fungi highlight numerous aspects of the relation between food and fungi including spoilage and fermentation [7,8].

MATERIALS AND METHODS

Sample Collection

Samples of packaged and unpackaged powdered milk, corn flour and soybean flour were randomly collected from Ekeonuwa market in Owerri Municipal using aseptic measures. Samples were transported in a sterile polyethylene bag to the Microbiology Laboratory, Imo State University, Owerri for immediate analysis.

Sterilization of Materials

The glass wares (Test tubes, conical flasks, beakers and Petri-dishes) were washed with soapy water and rinsed with distilled water; they were allowed to air dry and wrapped with Kraft paper and were further sterilized in a hot air oven at 180°C for 1hour and stored at 4°C.

Isolation of Fungi

Sabouraud Dextrose Agar (SDA) were prepared according to Manufacturer's instruction, and sterilized by autoclaving at 121°C for 15 min.

10 g of the collected samples were homogenized in 90ml of sterile distilled water. 1ml was pipetted from the stock solution and was transferred into a test tube containing 9ml of sterile distilled water and was shaked gently. The test tube was labeled 10^{-1}. From this test tube (10^{-1}), 1ml was taken using a fresh pipette and was transferred to a second test tube labeled 10^{-2}, subsequent dilutions followed up to 10^{-10} dilution factor, 1ml was removed from the 10^{-10} making all test tube 9mls.

1 ml of the 10^{-4} dilution factors of the homogenate was plated using pour plate technique. The samples were suspended into sterile Sabouraud Dextrose agar. Petri-dishes and molten agar was cooled at approximately 40-45ºC before been poured; the plates were rotated anticlockwise and clockwise direction for even distribution of the inoculums. The agar was allowed to solidify; the plates were inverted and incubated at room temperature (25ºC) for 4-5 days. Macroscopic observation of the cultural characteristics such as size, shape, color and consistency of the colonial growth was noted and subculture was made at the end of each incubation period to obtain pure culture of the colonies isolated. This was carried out on Sabouraud Dextrose agar.

Fungi Enumeration

At end of the incubation periods, the counts for each plate were counted and then were expressed as colony forming unit per ml of the sample (Cfu/ml). It was achieved by dividing the plate in to four, and then colonies were counted for one side and multiplied by four. Numbers of colonies depended on their size: typically, from 30 to 300 was appropriate on a standard 10cm Petri dish. CFU/ml was mathematically expressed as:

$$\text{number of colonies counted x dilution factorx 1ml}$$

Identification and Characterization of Fungi Species

The resulting cultures were identified to species level based on cultural and morphological characteristics like colony diameter, colony colour on agar and reverse, colony texture and zonation. Morphological features were studied under the microscope using lactophenol cotton blue stain and the major and remarkable microscopic features that were considered are conidiophores, conidial shape, phialides and metulae, presence and shape of vesicles. For microscopic characterization, slide cultures of the isolates were prepared and incubated in moist chambers at 28°C for 5 days before observation under a light microscope.

Lactophenol Cotton Blue Stain

Lactophenol Cotton Blue Stain is formulated with lactophenol, which serves as a mounting fluid, and cotton blue. Organisms suspended in the stain are killed due to the presence of phenol. The high concentration of the phenol deactivates lytic cellular enzymes thus the cells do not lyse. Cotton blue is an acid dye that stains the chitin present in the cell walls of fungi. Place a drop of Lactophenol Cotton Blue Stain in the center of a clean slide. Remove a fragment of the fungus colony 2-3mm from the colony edge using an inoculating or teasing needle or MycoMount™ adhesive strips (Cat. no. MM40). Place the fragment in the drop of stain and tease gently. Apply a coverslip. Do not push down or tap the cover slip as this may dislodge the conidia from the conidiophores. Examine the preparation under low and high, dry magnification for the presence of characteristic mycelia and fruiting structures. Consult appropriate references for diagnostic features of fungi isolated in clinical and non-clinical specimens.

RESULT

Table 3.1 shows the enumerated fungi load on packed and unpacked milk, corn flour and soybean flour. The packed milk has no fungi growth, the packed corn flour had a total fungi count of 3.1 x 10^4 Cfu/g, while soya bean flour had a total fungi count of 2.9 x 10^4 Cfu/g. However, the unpacked milk had a total fungi count of 3.2 x 10^5 cfu/g, corn flour had 3.7 x 10^5 cfu/g and soya bean had 3.0 x 10^5 cfu/g.

Table 3.1: Enumerated fungi load on packed and unpacked milk, corn flour and soybean flour

	Total Fungi Count (Cfu/g)		
	Milk	Corn flour	Soya bean flour
Packed	NG	3.1 x 10^4	2.9 x 10^4
Unpacked	3.2 x 10^5	3.7 x 10^5	3.0 x 10^5

Table 3.2 shows the isolated colonial morphology and microscopic morphology fungi species identified from the lactophenol cotton blue wet mount preparation. A total of four (4) fungi were packed and unpacked milk, corn flour and soybean flour are *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp. and *Penicillium* spp.

Table 3.2: Colonial and microscopic morphology of fungi isolated from packed and unpacked milk, corn flour and soybean flour

Colonial morphology	Microscopic morphology	Probable organism
Initially white to yellow but becomes distinctly black as colony develops	The vesicle of the conidiospores is large and globase, bearing two series of sterigmata over its entire surface. The conidia are brown to black and rough walled.	*Aspergilus* spp.
Colony forms grey to whitish greenish yellow coloration	Conidiospores bearing conidia with brush like spores	*Penicillium* spp.
Cotton-like, white in color to gray	Simple and form apical, globular sporangia that are supported and elevated by a column-shaped columella.	*Rhizopus* spp.
White cotton-like with tangled purple mycelia	Clear and non-pigmented clear septate with short conidiospores	*Fusarium* spp.

Table 3.3 shows the percentage distribution of the fungi isolated from packed and unpacked milk, corn flour and soybean flour. *Aspergillus* spp. was found to have the highest percentage occurrence of 35.7%, *Penicillium* spp. had 28.6%, *Rhizopus* spp. had 21.4% while *Fusarium* spp. had the least percentage of 14.3%.

Table 3.3: Percentage distribution of the fungi isolated from packed and unpacked milk, corn flour and soybean flour

Fungi isolates	Packed milk	Packed corn flour	Packed soybean flour	Unpacked milk	Unpacked corn flour	Unpacked soybean flour	Total Percentage (%)
Aspergilus spp.	-	+	+	+	+	+	35.7
Penicillium spp.	-	+	-	+	+	+	28.6
Rhizopus spp.	-	-	+	-	+	+	21.4
Fusarium spp.	-	+	-	-	+	-	14.3
Total	0	3	2	2	4	3	14(100)

DISCUSSION

Isolation and identification of fungi from packed and unpacked milk, corn flour and soya bean were studied. The findings of this study showed the presence of fungi contamination in these food products except for packed milk which had no fungi growth. The packed corn flour had a total fungi count of 3.1 x 10^4 Cfu/g, while soya bean flour had a total fungi

count of 2.9 x 10^4 Cfu/g. However, the unpacked milk had a total fungi count of 3.2 x 10^5 cfu/g, corn flour had 3.7 x 10^5 cfu/g and soya bean had 3.0 x 10^5 cfu/g. A higher fungi load count was observed in the unpacked samples. This could be attributed to the fact that these samples are easily expose to microbial contamination which can occur through air exposure, handling processes, utensils used [9,10].

Also, four genera of fungi were identified in this study. These are; Aspergillus, Rhizopus, Mucor and Penicillium. The isolation of these fungi confirmed the contamination of these samples except for packed milk sample which yielded no fungal growth. These food products were possibly contaminated by either the biotic or the abiotic factors which appeared to be one of the major factors that support fungal growth in food products [11]

The corn flour was more contaminated than other samples cultured because they were placed in exposed containers. Also, storage facilities such as sacks, polythene bags and natural fiber, which are air-tight being used by the traders in the market (Personal observation) for storage of all the varieties might have encouraged the fungal growth. This is because they can lead to continuous increase in humidity and temperature of the food product, which consequently favours fungal growth as reported by [12,13]. The most common fungus causing spoilage of food product is the *Aspergillus* spp. which was identified from the samples collected which showed the highest occurrence in this research. Moreover, food products can encounter fungal infestation by influences from outside environment, such as insect's infestation, wound and presence of foreign matter such as sand, dust and debris among others. Thus, some of the identified fungal species could have come from any of these sources. Similarly, the constant exposure of food products to the outside environment at the time of sales could have aided in deposition of the fungal spores on them [14]. Therefore, spores can germinate on the food products when temperature and humidity trigger the growth processes. Damage by insects has also been known to provide entry points for fungal infection [15,16] and aid in their rapid spread. Hence, presence of insects may under certain critical circumstances be quite essential for establishment of infection [18,19]. While several fungal species cause spoilage of food products worldwide, it is also noteworthy that presence of this known organisms isolated from this samples, are known to produce different mycotoxins like the ochratoxin, neurotoxin and aflatoxin. This mycotoxin may cause serious mycotoxicosis in man and in animal if produced in these food products tested [20,21,22]. Example *Penicillium* spp. is known to produces neurotoxin. The extent to which neurotoxin affect nerve function depends on the toxicity of the substance either by ingestion or by inhalation depending on the individual age and immune status. This toxin can have long lasting effect by causing neurons to malfunction or by disrupting interneuron communication which may eventually lead to paralysis or death [23, 24]. The ochratoxin is a naturally occurring foodborne mycotoxin found in a wide variety of agricultural products that can be produced by several fungal specie and genera like *Penicillium* or the *Aspergillus* specie. Ochratoxin (OTA) causes nephrotoxicity and renal tumors in different animal species [25]. However, health effect has linked OTA exposure with human disease known as Balkan endemic nephropathy (BEN) and chronic intestinal nephropathy (CIN) as well as other renal diseases. The generally most common and deadly mycotoxin produce is the aflatoxin known to be produced by *Aspergillus* species. *Aspergillus* infections have grown in importance in the last years.

CONCLUSION

From this research carried out species of *Aspergillus, Fusarium, Penicillium* and *Rhizopus* were isolated from the food samples tested. Some species of the fungi isolated are known to produce toxins like Aspergillus, Fusarium and Penicillium. Recovery of these fungi from this research shows that there is fear of consumption of mycotoxins because of their serious health implication, as they can be highly toxic and carcinogenic, thus rendering the food products unfit for human and animal consumption. Hence, it is important that contaminated food products should be sorted and eliminated to avoid re-infection. This will help to reduce the rate of mycoses.

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