

Mycological Quality of *Rastrineobola argentea* (Silver Cyprinid Fish) Sold in Markets in Owerri Metropolis, Nigeria

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Abstract

Fungi isolated from sun dried *Rastrineobola argentea* are associated with deterioration of the fish and is of public health importance. These may be as a result of inadequate sun drying, handling and storage, thus, the mycological quality of *R. argentea* sold in markets in Owerri metropolis was studied. A total of five markets were studied from which fifty retailers were sampled randomly and systematically. A sample of 20 g of *R. argentea* was collected from each participant. Samples were cultured on Sabourand dextrose agar to enumerate and identify the fungal isolates using cultural and microscopic methods. Analysis of variance (ANOVA) was used to determine the differences in the succession of fungi between and within markets at 5% level of significance. The results obtained showed that five fungal genera were isolated. These were; *Aspergillus* species 19 (44%), *Candida* species 11 (18%), *Penicillium* species 9 (28%), *Rhizopus* species 6 (18%) and *Mucor* species 5 (10%). The presence of mycotoxin producing species such as *Aspergillus* and *Penicillium* in the samples poses a real health risk through consumption or directly through contact with the fish products and therefore measures should be taken to improve their quality.

Keywords: *Mycological Quality, Rastrineobola argentea, Mycotoxin, Public Health, Consumption.*

Introduction

Rastrineobola argentea is a small, zooplanktivorous cyprinid fish from Lake Victoria and Lake Kyoga, East Africa [1]. It is a relatively cheap source of animal protein as nourishment for man and livestock as well as being the second most commercially important fishery of Lake Victoria. The fish constitutes a major source of protein in the diet of many people in developing countries like Nigeria. The fish is sun-dried to reduce post-harvest deterioration and to provide a microbiologically stable product at a reasonable cost. *R. argentea* that is properly dried can be

stored for up to two years without getting spoiled [2]. Traditionally, *R. argentea* are dried by laying the fish on the ground. This makes it contaminated with sand and animal waste thus loading it with bacteria and fungi [3]. This lowers the quality of the dried products as well as income. Efforts to improve the drying of *R. argentea* in the Lake Victoria region have been the introduction of racks. However, infestation by insects, aerial contamination and rain remain a problem during rack drying [4]. A further improvement of drying with racks is the use of solar dryers where drying in the enclosed chamber prevents insect infestation and rain, thus fish is dried faster and more hygienically [5].

However, the handling and storage quality of sun dried fish is reduced by the colonization of fungi that can grow even with minimal water content and makes it unfit for human consumption. When *R. argentea* is not stored properly, it absorbs moisture from the environment. This creates a favorable condition for the growth of fungi that cause rapid post-harvest deterioration of dried *R. argentea* [6]. Fungal contamination could precipitate public health concern and economic loss in terms of fish spoilage. Thus, this study was undertaken to evaluate the mycological quality of *R. argentea* sold in retail markets in Owerri, Nigeria.

Materials and Methods

Sample Collection

A total of fifty freshly sun dried *R. argentea* samples in consumer packages of 20g each were purchased from five markets in Owerri metropolis. The markets studied included: Ekeonuwa, Relief, New Owerri (Rochas), Amakohia and World Bank in Owerri metropolis. The samples were packaged aseptically in sterile polythene bags, labeled and immediately transported to the Microbiology Laboratory of Imo State University, Owerri for mycological analysis.

Mycological Assays

Mycological assays used included; enumeration

technique, isolation and identification.

Enumeration Technique:

Fungi were enumerated and isolated by pour plate method and growth on Sabourand Dextrose Agar (SDA) (Himedia, India) supplemented with 0.05 mg/ml chloramphenicol as described by [7]. Serial dilutions of 10^{-1} to 10^{-4} were prepared by diluting 1 g of each ground fish sample into 10 ml of sterile distilled water. A 1 ml aliquot from each of the dilutions was inoculated on Petri dishes and freshly prepared SDA was added. The plates were incubated at 25°C for 72 h. After incubation, colonies were counted using the colony counter and recorded in colony forming units per gram (CFU/g) of fish sample.

Isolation and Identification of Fungal Isolates:

From the fungal growth on primary cultures, fungal isolates were sub-cultured onto freshly prepared SDA without antibiotics. Fungal isolates were identified by macroscopic and microscopic characteristics of the colonies using descriptions of [7, 8] and [9].

Data Analysis

Analysis of variance (ANOVA) was used to determine the differences in the succession of fungi between and within markets at 5% level of significance.

Results

Enumeration of Fungi

After 72 h incubation, the fungal count ranged from 3.8×10^4 CFU/g to 1.8×10^5 CFU/g. The fungal count of sun dried *R. argentea* sold in retail markets in Owerri was significantly higher in three markets (Table 1). New Owerri market had the highest count (1.8×10^5 CFU/g) whereas Amakohia market had the lowest (3.8×10^4 CFU/g). Statistical analysis using ANOVA, indicated significant difference between the markets ($p < 0.05$).

Table 1: Total fungal count (TFC) of sun dried *R. argentea* in different retail markets

Market	Plate count	Dilution factor	TFC (CFU/g)
Ekeonuwa	76	10^{-3}	7.6×10^4
Relief	85	10^{-3}	8.5×10^4
New Owerri	180	10^{-3}	1.8×10^5
Amakohia	38	10^{-3}	3.8×10^4
World Bank	50	10^{-3}	5.0×10^4

Isolation and Identification of Fungi Associated with *Rastrineobola argentea*

Five fungal genera were recovered from the study. These were; *Aspergillus* species 19 (38%), *Candida* species 11 (22%), *Penicillium* species 9 (18%), *Rhizopus* species 6 (12%) and *Mucor* species 5

(10%) as presented in Table 2. *Aspergillus* species were the most frequently isolated whereas *Mucor* species were the least isolated.

Table 2: Percentage occurrence of fungi isolated

Isolate	Frequency of occurrence	Percentage occurrence
<i>Aspergillus</i> species	19	38
<i>Candida</i> species	11	22
<i>Penicillium</i> species	9	18
<i>Rhizopus</i> species	6	12
<i>Mucor</i> species	5	10
Total	50	100

Distribution of Fungal species in the different markets

New Owerri market had the highest distribution of fungal species 17 (34%) while samples from Amakohia market had the least distribution of fungal species 5 (10%). Relief market had 12 (24%), Ekeonuwa 9 (18%) and World Bank 7 (14%). *Aspergillus* and *Candida* species was present in all the samples collected from the various markets (Table 3).

Table 3: Distribution of fungal species isolated from sun dried *R. argentea* in different retail markets

Isolate	A	B	C	D	E	Total
<i>Aspergillus</i>	4(8%)	3(6%)	8(16%)	2(4%)	2(4%)	19(38%)
<i>Candida</i>	2(4%)	2(4%)	4(8%)	1(2%)	2(4%)	11(22%)
<i>Penicillium</i>	1(2%)	5(10%)	1(2%)	- (0%)	2(4%)	9(18%)
<i>Rhizopus</i>	- (0%)	1(2%)	3(6%)	2(4%)	- (0%)	6 (12%)
<i>Mucor</i>	2 (4%)	1(2%)	1(2%)	- (0%)	1(2%)	5 (10%)
Total	9(18%)	12(24%)	17(34%)	5(10%)	7(14%)	50(100%)

Key: A= Ekeonuwa, B= Relief, C= New Owerri, D= Amakohia, E= World Bank

Discussion

Fungal contamination was observed at various quantity in all fifty sun dried *R. argentea* samples purchased from various markets in Owerri. New Owerri market had the highest fungal count. This would imply that the sun dried *R. argentea* sold in various markets in Owerri is of low microbial quality and does not comply with standards. The results of the present study revealed that different fungal species were isolated from the fish samples. The result concurs with the findings [10] who reported that several species of yeasts and *Aspergillus* produce toxic substances which if consumed will cause health problems. A study carried out in Akwa Ibom State, Nigeria on the mycoflora of smoked dried fish indicated that *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus fumigatus*., *Mucor* sp. *Penicillium* sp. and *Rhizopus* sp. were associated with the dried fish [11]. This is in agreement with the findings of this study except for *Absidia* sp. and

Cladosporium sp which was not isolated from this study. [11] further observed that *Aspergillus* sp. and *Rhizopus* sp. were the most isolated. This agrees with the findings of this study which show that *Aspergillus* sp was the most isolated fungi, indicating their ubiquitous nature [12]. However, the findings of this study differ with those of [13] who reported that *Mucor* sp. was the most isolated fungi from stockfish.

Contamination of the fish samples by *Aspergillus* sp. and *Candida* sp. was common among the markets. The occurrence of *Aspergillus* sp. and *Candida* sp. from all the markets depicts that conditions favourable for the growth of these fungi are present in the study areas. The presence of these fungal species could also be due to poor hygiene by the fish handlers from the fishing point to the selling point. This has been reaffirmed by [14] who has asserted that fishes are prone to microbial attack due to unhygienic methods of handling, transportation and storage. During sun drying, the materials used and the overloading of the fishes on trays leads to improper processing which in turn encourages fungal contamination. This has been noted by [15] who has explained that the use of unhygienic fishing materials and the dirty clothes worn by the fishermen and other fish handlers encourage fungal contamination of the fish. Poor storage of the fish could be another cause of fungal contamination. For instance the sack bags the retailers used for storing the fish were placed on the ground and in a congested store. This was also observed by [16] who carried out a research on microbiological quality of *R. argentea* sold in Kisumu. Their findings indicated that the *R. argentea* that was stored in dirty baskets and in damp and dirty small stores at Kibuye market had high microbial contamination. The findings of this study indicate that during storage of sun dried *R. argentea*, good storage practices were not adhered to by the retailers. The store was not well ventilated and pests could easily gain access into it. This is contrary to the recommendations on environmental and public health in America that requires dried foods to be stored in rooms that are cool, dry and well ventilated with temperatures between 50°F and 70°F [6].

Conclusions

The study showed that all fifty sun dried *R. argentea* samples purchased from various markets in Owerri were contaminated with fungi at various quantities. The results of the present study also revealed that *Aspergillus* sp., *Candida* sp., *Penicillium* sp., *Rhizopus* sp. and *Mucor* sp. were isolated from the fish samples. Contamination of the fish samples by *Aspergillus* sp. and *Candida* sp. was common among the markets. The isolation of fungal species from sun dried *R. argentea* samples in the study poses a real health risk through

consumption or directly through contact with the fish products.

Recommendations

1. Public health workers should create awareness for the need to institute Good hygiene practices (GHP) and hazard analysis critical control point (HACCP) in fish processing and distribution.
2. Wholesalers and retailers of *R. argentea* should observe both personal and environmental hygiene when handling, storing and displaying the fish for sale.
3. Consumers of *R. argentea* should be encouraged to buy it from retailers whose stock turnover is high.
4. Those who buy *R. argentea* from the fishermen on the shores of Lake Victoria should be monitored by the public health officers in the respective areas to ensure that they dry it to the recommended moisture before selling it.

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