

MICROFUNGI ASSOCIATED WITH SUN DRIED *Rastrineobola argentea* SOLD IN GUCHA SOUTH DISTRICT IN KENYA

Abstract

The mycoflora of sun dried *Rastrineobola argentea* are associated with deterioration of the fish during sun drying, handling and storage in Gucha South district. A total of six markets were studied from which five wholesalers and thirty retailers were sampled randomly and systematically. A sample of 50 g of *Rastrineobola argentea* was collected from each participant during the period between September and December 2012. Samples were cultured on dichloran 18% glycerol (DG 18) agar to identify the fungi growing on the dried *Rastrineobola argentea*. The results obtained showed that thirteen different fungal species were isolated. These were; *Absidia* spp. (4.05%), *Aureobasidium* spp. (1.36%), *Alternaria* spp. (1.36 %), *Cladosporium* spp. (2.70 %), *Aspergillus niger* (17.57 %), *Aspergillus flavus* (9.46%), *Aspergillus fumigatus* (10.81%), *Fusarium* spp. (2.70 %), *Trichoderma* spp. (2.70 %), *Rhizopus* spp. (29.73 %), Yeasts (5.40 %), *Penicillium* spp. (6.76 %) and *Mucor* spp. (5.40%). The data obtained from the study showed that 83.79 % of all the isolates were toxin producing. The presence of mycotoxin producing species in the samples shows that the fish sold in Gucha South district could be a threat to the health of the consumers and therefore measures should be taken to improve their quality.

Key words

Microfungi, Associated, *Rastrineobola argentea*, Sun dried, Gucha South

Background Information

Fish constitute a major source of protein in the diet of many people in the developing countries where Kenya is included. Microfungi are microorganisms that can withstand very low water concentrations. This enables them to grow on substrates where most other microorganisms do not grow, for instance on sun dried *Rastrineobola argentea*. *Rastrineobola argentea* (Omena) is a major source of proteins in many rural areas in Kenya. From the community survey, it has been shown that the commonly consumed fish is *Rastrineobola argentea* (Omena) because it is readily availability in many markets in Kenya (FAO, 2007). This type of fish is cheap compared to Tilapia or Nile Perch and is easily treated by drying as a preservation technique. This makes it

stay for a long period of time without going bad (Mozola, 2001). For instance, *Rastrineobola argentea* that is properly dried can be stored for up to two years without getting spoiled while tilapia can hardly be stored for a month on smoking (Ofulla, 2007). *Rastrineobola argentea* now ranks second to the Nile perch among fish catches from Lake Victoria (Sablani, 2009).

Rastrineobola argentea (Omena) also known as fresh water sardine comprises 44% of total catch of fish from Lake Victoria on the Kenyan side. Dried form of this fish is the most widespread commodity with a relatively long shelf life, and can be sold in small portions to meet needs of the rural poor (FAO, 2007). Traditionally, *R. argentea* are dried by laying the fish on the ground, resulting in their contamination with sand and animal waste thus bacteria and fungi (Richard, 2006). This lowers the quality of the dried products as well as income.

Materials and Methods

Fifty grams of samples from five wholesalers and thirty retailers were systematically and randomly sampled from six markets, namely Etago, Nyamaiya, Riosiri, Nyamarambe, Ikoba and Tabaka in Gucha South district in Kisii County. The samples were collected twice from the markets between the period of August and December 2012. The samples collected were put in polythene bags, sealed separately, labeled and transported to **Kenyatta University Microbiology Laboratory**. The samples were stored at ambient temperature until they were microbiologically examined. In addition, unstructured interview was carried out to determine the mode of storage, duration of storage, hygiene and methods used to ensure that the *R. argentea* did not deteriorate (Mugenda, 2003).

Mycological assays used included; enumeration techniques, isolation and identification which largely involved microscopy.

Enumeration technique

i) Dilution and pour plate

Pour plate technique was used in quantifying and detection of microfungi (Pitt *et al.*, 2009) in crushed dried *Rastrineobola argentea*. Briefly, twenty grams of fish was soaked for ten minutes in 200 ml 0.1% pepton water and blended for five minutes (King *et al.*, 1998, Hocking, 2006). In addition, 20 gm of dry *Rastrineobola argentea* was ground to a powder using a blender with a mill. Serial dilutions were made by dissolving one gram in 9 ml (1:10) of peptone water. This was then serially diluted by transferring 1ml of the mixture into 9 ml peptone water to make 1:100 dilutions (Jacquelyn, 2008). A dilution of 0.1 ml from each dilution was then inoculated onto DG18 agar and AFPA agar and incubated at 30°C for 3 to 5 days (Nester *et al.*, 2007). The plates with colonies between 30 and 300 were enumerated in form of colony forming units (CFU). Those with less than 30 colonies were considered too few while those with more than 300 were categorized as numerous (Talaro, 2009).

ii) Direct Plating

The dried fish was cut into small pieces under sterile conditions. The pieces were surface sterilized with 5 % chlorine solution (Pitt *et al.*, 2009). After sterilization, the particles were rinsed in sterile water five times. The **inocula** were then inoculated aseptically onto solid *Aspergillus flavus* and *parasiticus* agar (AFPA) at a rate of 6-10 particles per plate depending on the size of the particles. The preparations were then incubated at 30° C for up to 7 days with

daily monitoring. After incubation the plates were examined visually, the number of infected particles counted and the results expressed as percentage.

iii) Isolation and identification of fungal isolates

From the fungal growth on primary cultures, fungal isolates were sub-cultured onto a fresh medium of DG18, MY50G and AFPA to get distinct colonies (Pitt *et al.*, 1997). The obtained pure isolates were then inoculated onto a slant of MY50G and DG18 and incubated at 30° C for further identification (Hocking, 2006). The mould was inoculated near the center of the slant to permit the best colony development and sporulation.

Taxonomic identification of the genus was carried out according to macro and microscopic characteristics of the colonies using identification keys by Barnett (1998), Pitt and Hocking (1997) and Samson *et al.* (1996). *Aspergillus flavus* and *Aspergillus parasiticus* were identified by their colors after growing in a selective medium; *Aspergillus flavus* and *parasiticus* agar (Copetti *et al.*, 2009).

Results

Thirteen different fungal species namely; *Absidia* sp., *Aureobasidium* sp., *Alternaria* sp., *Cladosporium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium* sp., *Trichoderma* sp., *Rhizopus* sp., Yeasts, *Penicillium* sp. and *Mucor* sp. were isolated from the samples of sun dried *R. argentea*. These fungal isolates were identified up to the genus level (plates 1a, 1b and 2).



Plate 1a: *Rhizopus* sp. as seen under the microscope



Plate 1b: Colony of *Rhizopus* sp.



Plate 2: Colony of *Fusarium* sp.

Frequency of fungal species

All the samples collected contained more than one **fungus** (Table 1). Riosiri had the lowest number **with** four different isolates while samples from Nyamaiya market contained the largest number of different fungal species which was twenty four out of seventy four isolates obtained from all the collected samples. Tabaka market had 8 isolates, Etago 17, Nyamarambe 11 and Ikoba 10.

Aureobasidium sp. and *Alternaria* sp. isolates were the least isolated, that is, one isolate each while *Rhizopus* sp. isolates were the most frequently isolated and had a total of twenty two isolates. *Rhizopus* sp. was present in all the samples collected from the various markets. *Fusarium* sp., *Trichoderma* sp. and *Cladosporium* sp. had an occurrence rate of 2 each. *Absidia* sp. had 3, *A. niger* 13 while Yeasts and *Mucor* sp. had an occurrence rate of 4 each out of 74 isolates. *Penicillium* sp. had 5, *A. flavus* 7 while *A. fumigatus* had an occurrence rate of 8 out of 74 isolates (Table 1).

Table 1: The rate of occurrence of fungal isolates from different markets

Rate of Occurrence of Fungal Isolates							
Fungus	Riosiri	Tabaka	Nyamaiya	Etago	Nyamarambe	Ikoba	Total
<i>Absidia</i> spp.	-	2	-	1	-	-	3
<i>Aurebasidium</i> sp.	-	-	1	-	-	-	1
<i>Alternaria</i> spp.	-	-	1	-	-	-	1
<i>Cladosporium</i> spp.	-	-	1	1	-	-	2
<i>A. niger</i>	-	1	6	2	3	1	13
<i>A. flavus</i>	-	1	2	3	-	1	7
<i>A. fumigatus</i>	1	-	3	-	2	2	8
<i>Fusarium</i> spp.	-	-	-	1	-	1	2
<i>Trichoderma</i> spp.	-	1	-	-	1	-	2
<i>Mucor</i> spp.	1	-	2	-	1	-	4

Yeasts	1	-	1	2	-	-	4
<i>Penicillium</i> spp.	-	-	2	1	2	-	5
<i>Rhizopus</i> spp.	1	3	5	6	2	5	22
Total	4	8	24	17	11	10	74

KEY: - - Absence of fungal isolate, A – *Aspergillus*, Sp. – Species, numbers represent occurrence rate of the isolates out of a total of 74.

After sampling twice, the percentage frequency of the fungal isolates ranged from an average of 1.5-25.0. *Rhizopus* sp. had the highest frequency of 25.0 percent while *Aureobasidium* sp., *Trichoderma* sp. and *Alternaria* sp. had the lowest frequency of 1.5 percent. *Aspergillus flavus* had a frequency of 17.0 percent, *Absidia* sp. 4.0, yeasts and *Mucor* sp. 4.5, *Cladosporium* sp. 2.5, *Penicillium* sp. 8.0, *Aspergillus niger* 18.5, *Aspergillus fumigatus* 11.5 and *Fusarium* sp. 2.0 (Fig. 1).

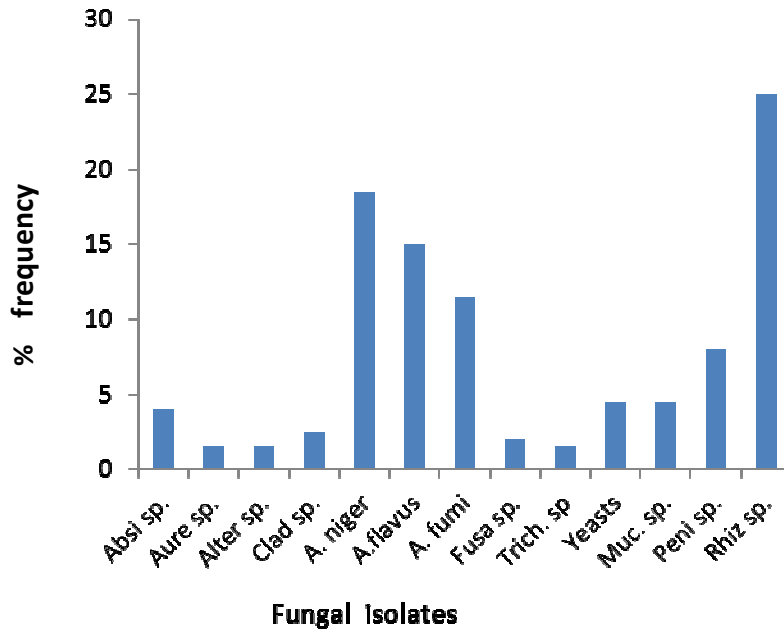


Figure 1: Percentage frequency of fungal isolates from different markets in Gucha South district

KEY: Absi sp.- *Absidia* species, Aure sp.- *Aureobasidium* species., Alter sp.- *Alternaria* species, Clad sp.- *Cladosporium* species, A. niger- *Aspergillus niger*, A. flavus- *Aspergillus flavus*, A. fumi- *Aspergillus fumigatus*, Fusa sp.- *Fusarium* species, Trich. sp- *Trichoderma* species, Muc. sp.- *Mucor* species, Peni sp.- *Penicillium* species, Rhiz sp.- *Rhizopus* species.

Discussion

The findings of this study indicate that thirteen fungal isolates were associated with the sun dried *R. argentea*. These were *Absidia* sp., *Aurebasidium* sp., *Alternaria* sp., *Cladosporium* sp., *A. niger*, *A. flavus*, *A. fumigatus*, *Fusarium* sp., *Trichoderma* sp., *Rhizopus stolonifer*, *Yeasts*, *Penicillium* sp. and *Mucor* sp. These findings imply that consumption of the sun dried *R. argentea* could pose serious health problems to the consumers. This has been explained by Wood (1992) who has observed that several species of yeasts and *Aspergillus* are known to produce toxic substances which if consumed will cause health problems. A study carried out in Uyo state, Nigeria by Bukola *et al.* (2008) on the mycoflora of smoked dried fish indicated that *Aspergillus*

niger, *Aspergillus flavus*, *Aspergillus tereus*, *Aspergillus fumigatus*, *Absidia* sp., *Mucor* sp., *Cladosporium* sp., *Penicillium* sp. and *Rhizopus* sp. were associated with the smoked fish. This concurs with the findings of this study except for *Aspergillus tereus* which was not isolated during the study. Bukola *et al.* (2008) further observed that *Aspergillus* sp. and *Rhizopus* sp. were the most isolated. This agrees with the findings of this study which show that *Rhizopus* and *Aspergillus niger* are the most isolated indicating their ubiquitous nature (Kaferstein, 2005). However, the findings of this study differ with those of Junaid (2010) who found out that *Mucor* sp. was the most isolated from stock fish and Copetti (2009) who found out that *Eurotium* sp. was the most isolated from dry animal feeds made from fish. These findings imply that consumption of contaminated sun dried *R. argentea* could pose serious health problems as *Aspergillus* sp. are known to produce mycotoxins, the most hazardous being aflatoxin. For instance, aflatoxins have been implicated in cases of acute hepatitis in man and are also known to be carcinogenic, causing hepatoma (Eaton *et al.*, 1994).

The presence of the thirteen fungal isolates could be due to poor handling of the fish right from the harvesting stage to the selling point. In artisanal fishery, freshly caught fish are covered with damp sacks and at times, they are mixed with wet grass or water weeds to reduce the temperature (Owaga, 2009). Fish treated this way is prone to contamination with microorganisms such as bacteria and fungi as observed by Tobor (1992). This indicates that contamination and spoilage of fish starts right from the harvesting stage.

The presence of these fungal species in the sun dried *R. argentea*, could also be due to poor hygiene by the fish handlers from the fishing point to the selling point. This has been reaffirmed

by Eyo (1992) who has asserted that fishes are prone to microbial attack due to unhygienic methods of handling, transportation and storage. During the sun drying period, the sun drying materials used and the overloading of the fishes on trays leads to improper processing which in turn encourages fungal attack. This has been noted by Abila (2003) who has explained that the use of unhygienic fishing materials and the dirty clothes worn by the fishermen and other fish handlers encourage fungal attack on the fish.

Poor storage of *R. argentea* could be another cause of fungal contamination. For instance the ganny bags wholesalers and retailers used for storing the fish were placed on the ground and in a congested store. This was also observed by Sifuna *et al.* (2008) 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 wholesalers and 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 (Robert, 2005). Therefore appropriate handling and storage conditions are important in minimizing the growth of fungi on sun dried *R. argentea*. Good hygiene practices (GHP) and hazard analysis critical control point (HACCP) should also be made operational along the harvesting, processing and distribution line of *R. argentea*. This will ensure that the *R. argentea* is produced under hygienic conditions so as to address possible risks associated with contamination.

The environment where *R. argentea* are displayed in the market is not always hygienic and this is another avenue for microbial contamination. Very often, retailers displayed the sun dried *R. argentea* samples in open stands beside the gutter on refuse heaps and open sewage draining trenches. This encourages fungal attack and subsequent production of toxins.

The toxigenic fungi isolated from *R. argentea* during this study were *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium* sp. *Alternaria* sp. and *fusarium* sp. This concurs with the findings of a study conducted by Junaid (2010) in Jos Metropolis in Nigeria on mycotic contamination of stock fish. This also agrees with the findings by Makun (2010) where *Aspergillus fumigatus*, *Fusarium* sp., *Penicillium* sp. and *Mucor* sp. were detected in dried yam chips sold in Kogi state in Nigeria. The presence of these fungi in foods is of great concern. This is because *Aspergillus*, *Penicillium* and *Fusarium* species, produce aflatoxin B1 transformed into aflatoxin M1 found in the milk, as well as Ochratoxins, Zearalenone (Truce, 2007), Fumonisin B1, T-2 toxin, HT-2 toxin and deoxynivalenol (vomitoxin), which are of increasing concern in human health (Wild, 2010). This is because the growth of these commonly occurring filamentous fungi in foods may cause a variety of ill effects in humans (Wu, 2010), from allergic responses to immune suppression and cancer (Wild, 2010). For instance, aflatoxins are potent carcinogens and in association with hepatitis B virus are responsible for many thousands of human deaths per annum in non-industrialized tropical countries (Wugo, 2011).

This study showed that 7 % of the fungi isolated were *Penicillium* sp. while 38 % were *Aspergillus* sp. The findings of this study are not different from those obtained by Montagna (1998) who found out that *Aspergillus* and *Penicillium* were contaminants of commercial

yoghurt sold in Italy (Montagna, 1998). These species of fungi are known to produce ochratoxin A which has been labeled as carcinogenic and nephrotoxic (Krogh, 2010). It has been linked to tumors in human urinary tract (Aish, 2004) and can also accumulate in animal products like meat (Makun, 2010). This therefore implies that the growth of these fungi on sun dried *R. argentea* can lead to contamination of the fish with this toxin. This could pose a public health risk to consumers of *R. argentea* given that, wholesalers and retailers in the studied markets stored the fish poorly. The concentration of these fungi in the stored fish can increase to hazardous levels that could even lead to death. Therefore, *R. argentea* need to be stored safely and properly to prevent fungal growth risk from contamination and to ensure quality. Storage areas also need to be maintained clean regularly and checked for any signs of pest infection which could compromise food safety (Chartered Institute of Environmental Health, 2010).

The detection of *Fusarium* sp. in the fish during the study implies that there is a possibility of production of fumonisins, trichothecenes and zearalenone which are the mycotoxins associated with these fungi. This is because the moisture content in the sampled fish was higher than the recommended level for dried *R. argentea* and this could encourage rapid growth of *Fusarium* sp. on the fish resulting in increased production of the mycotoxins (Murphy, 2006). Fumonisin affect the nervous system and may cause cancer. Trichothecene is associated with chronic and fatal toxic effects while zearalenone causes negative effects on the reproductive system (Truce, 2007). These mycotoxins are more likely to be produced when there is poor method of food handling, storage and malnutrition (Shepard, 2008).

Therefore, since the sun dried *R. argentea* sold in South Gucha district contained these toxigenic fungi, the consumers of these fish are at risk of suffering from serious health problems associated with ingestion of mycotoxins especially when present in foods in sufficiently high levels (more than 20 ppb for aflatoxin) (Wu, 2010). Some of the toxic effects due to fungal metabolites range from acute (for example, liver or kidney deterioration), to chronic (for example, liver cancer), mutagenic, and teratogenic; with resulting symptoms ranging from skin irritation, immune suppression, birth defects, neurotoxicity, stunted growth in children and death (Murphy, 2006).

Ensuring that *R. argentea* is properly handled and stored is extremely crucial as this will go a long way in ensuring that the consumers of this type of fish are protected from exposure to mycotoxins. This will help reduce the effects and diseases related to mycotoxin consumption most of which are expressed in the long term.

Other fungal species detected during the study that are significant to public health were *Aureobasidium* sp., *Alternaria* sp., *Mucor* and *Cladosporium* sp. *Aureobasidium* sp. has a world-wide distribution and it is usually isolated as a saprophyte, occasionally from skin and nails. This implies that poor hygiene could lead to introduction of this fungus to sun dried *R. argentea*. The fungus has been reported as a rare causative agent of phaeohyphomycosis, mycotic keratitis and peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD).

The spores of *Alternaria* sp. and *Cladosporium* sp. isolated during the study can cause mold allergy symptoms when inhaled. These symptoms vary from one person to another and range from mild to severe. The mold spores and very fine hyphal fragments trigger allergic reactions

in about 20% of the adult population (Jackson, 2011). The reactions are due to protein and polysaccharide components of molds (Truce, 2007). This is because the small spores and fine hyphal fragments of the fungi easily penetrate deep into the respiratory tract. The mold allergy symptoms are of two types, the immediate hay-fever or asthma Type I and the delayed farmer's lung Type III. Type I allergy occurs only in allergic individuals and may be caused by many types of molds (Jackson, 2011). This therefore implies that allergic individuals exposed to sun dried *R. argentea* contaminated with these fungi are at risk of having their problem magnified.

Prolonged inhalation of spores and hyphal fragments of *Alternaria* sp. and *Cladosporium* sp. can cause mold allergy symptoms such as sneezing, nasal congestion and itchy or watery eyes in healthy individuals (Leschy, 2006). People who suffer from asthma may experience wheezing and shortness of breath. Therefore wholesalers, retailers and consumers of poorly dried and stored *R. argentea* can develop mold allergy related illnesses.

Controlling mold allergy means controlling one's exposure to mold. That is not always easy unless it is done by controlling mold growth on our environment and the foods we get exposed to (Jackson, 2011), for instance through drying fish to the recommended moisture content, proper handling and storage of fish. Hence controlling the growth of molds is vital in the reduction of mold allergies.

Conclusion

Sun dried *R. argentea* sold in South Gucha district is contaminated with fungi. Some of the fungi for example, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp. and *Fusarium* sp. are

known to produce mycotoxins that pose a threat to the health of individuals who consume them. The concentration of these fungi in some markets exceeded the recommended CFU/g according to Kenya Bureau of Standards (KEBS). Therefore some of the sun dried *R. argentea* consumed in South Gucha district is unfit for human consumption.

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Competing interests

The authors declare that they have no competing interests.

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