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Mycotic contamination of stockfish sold in Jos, Nigeria

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This study was aimed at isolating and identifying the fungi associated with stockfish contamination in Jos Metropolis. A total of 100 stockfish samples were randomly purchased from four markets namely, Terminus, Kwararafa, Katako and Gada biu in Jos town, Plateau State, Nigeria. The stockfish samples were assayed for fungal contamination and moisture content using standard procedures. All the stockfish samples were contaminated with fungi. Seven different fungi were found to be associated with the stockfish samples sold in the four different markets. The associated fungi were *Mucor Spp, Asergillus flavus, Trichophyton verrucosum, Aspergillus niger, Aspergillus fumigatus, Penicillin Spp and Rhizopus Spp.* It was observed that *Mucor Spp* had the highest rate of occurrence among the isolated fungi. The moisture content was between 6 - 27%. Results from the study are useful in developing and establishing public health standards as consumption of these fungi exposes the consumers to the probable toxic metabolites produced by the fungi.

Key words: Mycotic contamination stockfish, cod.

INTRODUCTION

Fish supplies a good balance of proteins, vitamins and minerals. It has relatively 10% calories content hence its role in nutrition is recognized (Akande and Tobor, 1992). Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas (Adeleye, 1992) . They are widely accepted on the menu card and form a much cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Adeleye, 1992). Fish flesh is tender due to bundles of muscle fibres which are held together by fibrous material when heated (Fagade, 1992). It is better digested than beef or other types of protein (Adebayo-Tayo et al., 2008).

Fish is an extremely perishable food. It begins to spoil as soon as it is caught perhaps even before it is taken out of the water. Spoilage proceeds as a series of complex enzymatic, bacterial and chemical changes that begin as soon as the fish dies. This is why the fish become soft and the smell becomes more noticeable (Carruthers, 1986). In Nigeria, fish is eaten fresh, preserved or processed (Adebayo – Tayo et al., 2008). Fish processing and preservation is carried out to slow down or prevent the enzymatic, bacterial and chemical deterioration to maintain the fish flesh (Carruthers, 1986). Food preservation can be achieved by the removal of water from the food items since the microbial deteriogens require moisture for active growth including enzymatic hydrolysis of the food components (Ogbonna, 1987). Water occurs naturally on the fish's body and so drying is one of the simplest ways to preserve fish. It works by removing water from the fish which prevents the growth of micro-organisms and decay. Drying food using the sun and wind to prevent spoilage has been known since ancient times, water is usually removed by evaporation (air drying, sun drying, smoking or wind drying) but in the case of freeze drying, food is first frozen and then water is removed by sublimation. Drying creates a hard outer layer helping to stop micro-organisms from entering the food (Wikipedia, 2009a). In other to prevent spoilage, the moisture content needs to be reduced to 25% or less. The percentage will depend on the oiliness of the fish or whether it has been salted (Facts, 2004).

Stockfish is unsalted fish especially cod dried by sun and wind on wooden racks on the foreshore called "Flakes" or in special drying houses (Wikipedia, 2009b).

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Cod is the common name for the genus of fish *Gadus* belonging to the family *Gadidae* and is also used in the common name of a variety of other fishes. It is a whitefish referring to several species of the pelagic deep water fish with fins particularly Cod (*Gadus morhua*), whiting *(Merluccins bilinearis*) haddock (*Melanogrammus aeglefinus*) but also hake (*urophycis*), Pollock (*Pollachins)* or others. Cod is sometimes eaten straight but often reconstituted. For centuries, it was preserved by drying as stockfish and clip fish and traded as a world commodity. During the drying, about 80% of the water in the fish disappears. The stockfish retained all the nutrients of the fresh fish only concentrated. It is best known to be one of the richest sources of protein with the important B vitamin, Iron and calcium. Cod is moist and flaky when cooked and is white in colour. It has a mild flavour, low fat content and a dense white flesh that flakes easily (Kurlansky, 1997).

Stockfish is popular in West Africa where it is used in many soups that complement the grain staples fufu and garri. The Bakweri, who are a fishing people of the English – speaking part of Cameroon use stockfish in flavouring their palmnut or banga soup which is eaten with a cocoyam pudding called Kwacoco. The name "Akporoko" for stockfish among the Annang of Nigeria refers to the sound the hard fish makes in the pot and literally translate as that which produces sound in the pot (Wikipedia, 2009b). Dried fish has a storage life of several years and thus, it gives fungi a greater opportunity to contaminate it. Fungi are omnipresent in the environment, being found wherever water, suitable organic nutrients and an appropriate temperature occur. They secrete enzymes outside their body structure and absorb the digested foods (Prescott et al., 1999). The growth of filamentous fungi in foods and food products results in waste and is costly as well as sometimes hazardous. Many different fungal species can spoil food product or produce mycotoxins or both (Anderson and Thrane, 2006). Mycotoxins are secondary metabolites produced by moulds that are capable of causing disease and death in humans and animals (Bennett and Klich, 2003). Drying to moisture content below 15% prevents the growth of many spoilage organisms while mould growth is only suppressed at 10% moisture content (Buere, 2005).

In a study of mycoflora of smoke dried fishes sold in Uyo, Eastern Nigeria by Adebayo-Tayo et al. (2008), twelve different fungi were found to be associated with the smoke – dried fish samples. The associated fungi were *Aspergillus flavus, Aspergillus tereus, Aspergillus fumigatus, Absidia sp, Rhizopus sp, Aspergillus niger, Mucor sp, Cladosporium Sp, Penicillin italiculum, P. viridatus, Candida tropicalis and Fusarium moniliformis.* Moulds may be present without producing any toxin (Bennett and Klich, 2003), but the presence of toxigenic fungi increases the risk for mycotoxin production (Jacobsen et al., 2008). Even though the fungus is no longer alive, while it was growing, if it produced a mycotoxin it will have poisoned the food (Wong, 2007). Mycotoxins greatly resist decomposition or being broken down in digestion so they remain in the food chain and even temperature treatments such as cooking and freezing do not destroy the mycotoxins. It has been proven that food items do carry residues of the toxins and thus it is certain that human beings are exposed to mycotoxins through contaminated food items among which fish is an important component (Adebayo-Tayo et al., 2008).

Justification

Stockfish, especially the heads are common in the markets. They are popular because of their rich taste and aroma and are sometimes eaten raw but mostly used in cooking most native soups. Moulds have been observed to grow on them and hence the need for this study to asses if they are known mycotoxin producing species.

MATERIALS AND METHODS

Samples and sampling sites

A total of one hundred stockfish samples used for this study were randomly purchased from four markets namely: Terminus, Kwararafa, Katako and Gada biu in Jos town, Plateau State, Nigeria. The samples were collected in sterile polyethylene bags.

Sample processing

About ten grams portion of each stockfish tissue was aseptically cut from the central portion using a sharp sterile scalpel blade and a forceps and transferred into the polyethylene bags.

Isolation of fungi

On each of the dried Sabouraud Dextrose Agar plates to which penicillin and streptomycin had been incorporated were inoculated in the processed samples using sterile forceps. The inoculated plates were incubated upright at 25˚C for 3 - 5 days. All observed colonies were subcultured to obtain pure cultures (ICFM, 2007).

Identification of fungi

The growth rate, colour, texture, colonial morphology and diffusible pigmentation of each sample were examined macroscopically. Tease mount using lactophenol cotton blue was adopted and microscopic features such as spore and hyphae morphology were observed and compared with the standard colour atlas as described by Ochei and Kolhatkar (2000).

Moisture content determination

Moisture content of 5 g- of each of the stockfish samples was determined by oven drying method as described by

Table 1. Sample distribution.

Market location	Number of samples
Terminus	20
Kwararafa	38
Katako	12
Gada Biu	30
Total	100

Table 2. Frequency of the fungi species Isolated from stockfish from the different markets.

Fungus	Terminus	Kwararafa	Katako	Gada biu	Total
Mucor Spp	19	30	12	24	85
Aspergillus Flavus	3	13	3	10	29
Trichophyton verrucosum	-	3	4	4	11
Aspergillus niger	2	2	-	3	7
Aspergillus fumigatus	-	1	-	2	3
Pencillium	-	2	-	-	2
Rhizopus Spp	-	2	-	-	2
Total	24	53	19	43	139

Table 3. Frequency of mixed growth of fungi species isolated from stockfish from the different markets.

Fungi	Terminus	Kwararafa	Katako	Gadabiu	Total
Mucor Spp/A. Flavus	3	4	1	4	12
Mucor Spp/T. verrucosum	-	3	4	2	9
A. Flavus/T. verrucosum	-	-	-	2	2
A. Flavus/Rhizopus Spp	-	2	-	-	2
A. Flavus/A. niger	-	2	-	-	2
A. Flavus/A. fumigatus/ A. niger	-	-	-	3	3
Mucor Spp/ A. Flavus/ A. niger	2	-	-	-	2
A. Flavus/A.fumigatus/Penicillium Spp	-	2	-	-	2
Total	5	13	5	11	34

Adebayo-Tayo et al. (2008).

RESULTS

Results obtained from this study showed that One hundred (100%) of the samples obtained were contaminated with fungi. Table 1 shows the sample distribution. Tables 2 and 3 show the frequency of the fungi species and the frequency of the mixed growth of fungi species isolated from the stockfish samples in the different markets. Table 4 shows the distribution of each of the fungi species isolated with *Mucor Spp* as the most dominant mycoflora 85 (61.2%) > *A. flavus* 29 (20.9%) > *Trichophyton verrucosum* 11 (7.9%) > *A. niger* 7 (5.0%) > *A. fumigatus* 3 (2.2%) > *Penicillium Spp* 2 (1.4%) = *Rhizopus Spp* 2 (1.4%). Table 5 shows the moisture content of the stockfish samples ranging from 6 - 27%.

DISCUSSION

Dried fish products still possess the largest volume on the processed seafood market among developing nations in the world today. Their finished forms can be packaged, stored and shipped economically which explains their long lasting presence especially in less developed areas of this world such as African nations and China. Nigeria has been importing large quantities of high grade stockfish not available locally from all over the world in the last few decades (Jason, 1695). Results from this study showed that all the stockfish samples were contaminated with fungi. Fungi produce spores which are moderately resistant to drying and therefore easily

Table 4. Distribution of fungi species isolated from the study.

Fungus	Number isolated	Percentage (%)
Mucor Spp	85	61.2
A. Flavus	29	20.9
T. verrucosum	11	7.9
A. niger	7	5.0
A. fumigatus	3	2.2
Pencillium Spp	2	1.4
Rhizopus Spp	2	1.4
Total	139	100

Table 5. Moisture content (%) of the stockfish samples.

Moisture content (%) range	Number of samples			
	Kwararafa	Gada biu	Terminus	Katako
6-10	3	8	2	1
11-15	15	16	15	5
16-20	12	6	3	1
21-27	8	_	_	5
Average	17.0	13.3	18.2	18.8
Highest	26	20	18	27
Lowest	6	7	6	10

implicated in the contamination and spoilage of dry and semi-dry materials. Stockfish has a low moisture content which makes it more susceptible to fungi action than bacteria (Holdsworth, 1971). This is also in agreement with the findings of Eaton and Groopman (1994) that moulds have the ability to survive harsh conditions and low moisture content (Ekundayo, 1984).

The associated fungi were *Mucor Spp* being the most dominant mycoflora followed by *A. niger*, *A fumigatus*, *Penicilium Spp* and *Rhizopus Spp* in decreasing sequential order. The distribution of these fungi probably results from the ubiquitous nature of fungi in agreement with Ibeh et al. (1991) and production of numerous air borne conidia which easily disperse by air movements and possibly insects. *Trichophyton verrucosum*, a dermatophyte which is a cosmopolitan zoophilic fungus with cattle as the usual habitat ranked third highest (7.9%) in the distribution of the associated fungi. This could be as a result of acquisition of fungus from the retailers since it is acquired by contact with contaminated soil or with infected animals or humans (Mitchell, 2007) where it affects the beard, neck, wrist and back of hands (Ochei and Kolhatkar, 2000) which are all exposed parts of the body. The other moulds isolated are common in the air and soil and have been linked with the production of various types of toxins under various conditions in agreement with Beatriz and Eliana (2000). *Aspergillus Spp* has been proven to produce aflatoxin and ochratoxin, *A. Penicillium Spp* produces aflatoxin and citrinin while *Rhizopus Spp* produces aflatoxin. Mari and Riccioli (2004) reported *A.niger* and *A. fumigatus* as being allergenic. The presence of these fungi in the stockfish samples might probably make the consumption of the stockfish hazardous to health as similarly reported by Adebayo – Tayo et al. (2008) because they might contain metabolites produced by the fungi. The potency of these metabolites is not affected by cooking and may cause severe or fatal damage to the liver and kidney (Mitchell, 2007).

The study also observed mixed growth of fungi in various combinations of two or three fungi from all the markets which could be as a result of the presence of competitive mycoflora that is, the associated growth of other moulds which influences fungal growth in stored products (Bennett and Klich, 2003). Results from the study also revealed a moisture content range of 6 - 27% with average values of 18, 26, 27 and 20% from Terminus, Kwararafa, Katako and Gada biu markers, respectively. Stockfish has a moisture content of less than 15% (Holdsworth, 1971) which differs from that of some of the stockfish samples. The high moisture content of these samples could be as a result of relative humidity which will allow unpackaged dried fish with initial low moisture content to take up sufficient water to allow mould growth (Buere, 2005). This also agreed with Gibson et al. (1994) findings that atmosphere

composition had a great impact on mould growth with humidity being the most important variable. Adventitious storage fungi grow at moisture contents of 15 - 20% in equilibrium with a relative humidity of 70 - 90%. When the relative humidity falls below 65% no growth occurs (Cockerell et al., 1971).

Furthermore, the rise in moisture content could also be as a result of the fact that fungi growing in food can raise the temperature in their immediate environment to 55˚C with concomitant increase in moisture content of the affected food as high as 20% (Cockerell et al., 1971). According to Smith et al. (1995), insect activity which decreases the host's immunity can also increase moisture content through condensation of moisture resulting from respiration. Sometimes in imported produce as in the case of stockfish, materials are infested by insects before stacking. If the core of the stack is infected from the start of the storage, then the heat of metabolism will raise the temperature and ultimately increase the moisture content in agreement with Cockerell et al. (1971). This also agrees with Bennett and Klich (2003) findings that mycotoxin problems were exacerbated whenever shipping, handling and storage practices are conducive to mould growth. During the storage of stockfish, good storage practices are not adhered by the wholesaler hence the stores are not well ventilated and pests can easily gain access. This is in agreement with the findings of Adebayo – Tayo et al. (2008).

Samples with low moisture contents were also contaminated which disagrees with Buere (2005) that mould growth is completely suppressed only at 10% moisture content and Smith et al. (1995) that quality in storage is preserved by the prevention of biological activity through adequate drying to less than 10% moisture content. The results of this study however agree with Detroy et al. (1971); Wilson and Payne (1994) findings that moisture content of food is closely related to the relative humidity. More so, *A. flavus* has been shown to produce toxins at relatively low moisture levels and during drought stress (Diener et al., 1987; Klich, 1987) . In summary the contamination of the samples could also be as a result of the unhygienic environment in which the stockfish are displayed in the market. This agrees with the report of Akande and Tobor (1992); Adebayo – Tayo et al. (2008). Very often, the stockfish are displayed in open baskets or on tables beside the gutter or refuse dumps and this encourages fungi attack and subsequent production of toxins.

Conclusion

Stockfish samples on sale in Jos metropolis were found to be contaminated with moulds which are known mycotoxin producing species. Although, moulds may be present without producing any toxin, the presence of the toxigenic fungi increases the risk for mycotoxin production. Mycotoxins from sufficient evidence from animal models and human epidemiological data pose an important danger to human and animal health because they are toxic to vertebrates and other animals in low concentrations. The implication of this report is that most consumers might have been consuming these metabolites and their prolonged intake may constitute a health hazard.

In other to demonstrate that a disease is a mycotoxicoses, it is necessary to show a dose- response relationship between the mycotoxin and the disease. Toxin detection and quantification was however not carried out due to unavailability of the reagents in this locality. The determination would have provided data for assessement if mycotoxins are indeed produced in the samples and if the produced quantify, falls within acceptable limits. Seventy seven countries are known to have regulations limiting mycotoxin levels (Bhatnagar et al., 2006).

Recommendation

Contamination occurs by invasion of toxigenic strains ubiquitously found before and during harvesting or processing or by improper storage thus the prevention of contamination is not an easy task. Proper control strategies include inhibiting fungal growth by the use of organic acids. The uses of fungistatic reagents such as potassium sorbate, propionic acid and gentian violet have been employed to prevent and control the growth of moulds. Intense insect activity often results in mould growth because of the increased moisture and temperature and so the prevention of mould contamination also depends on the successful control of insect infestation, determined by the following factors:

(i) The general hygiene of the store which determines whether or not insects can breed in the building,
(ii) The turnover of the goods which determines the length of storage, and
(iii) The way in which waste and odd lots are handled which determines whether or not large foci of infestation can develop in neglected produce.

The existence of hydrophilic toxins like fumonisins which are not soluble in organic solvents raises the spectre that there may be many other occult but toxic products of fungal metabolism that have not yet been discovered since they are difficult to study. It is therefore recommended that the possibility of producing mycotoxin detection systems designed for hydrophilic toxins can be considered.

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