Toxigenic Fungi and Mycotoxin Production in Maldive Fish (Smoked Dried Tuna Fish)

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Abstract

This is the first study on the mycological safety of “Maldive fish”, a smoked dried tuna product that is both economically and nutritionally important to the Maldives. The most obvious concern with this product is the effect of fungal contamination. The initial aim of the current study was therefore to determine if Maldive fish supports the growth of toxigenic fungi and production of mycotoxins.

The uncontrolled mycoflora on the product were characterised and related to the physiological parameters of the Maldive fish. Ninety six percent of the samples (n=25) were contaminated with one or more mycotoxigenic fungi with *Aspergillus flavus* (92%), *A. tamarii* (96%), *A. niger* (40%), *A. ochraceus* (12%) and *Penicillium citrinum* (60%) identified as the significant species. Subsequently, the potentially toxigenic isolates were screened for their corresponding mycotoxins aflatoxins, ochratoxin A (OTA), cyclopiazonic acid (CPA) and citrinin. A high proportion (72%) of isolates was able to produce toxic metabolites *in vitro* indicating possible contamination of the product with mycotoxins. Almost half (46%) of the *A. flavus* isolates were able to produce the potent carcinogen, aflatoxin B. All species on the surface were also found invading the product. The huge variability in a_w levels (0.951 to 0.720) of the samples would support growth of a wide range of species. Furthermore, the slightly acidic pH (5.65 to 6.68) and low salt content (1.48 to 4.29%) together with the high ambient temperatures of the Maldives were eminently suitable for fungal growth and mycotoxin production. Quantification of aflatoxins from the product revealed two of the 25 samples to be contaminated above the legal limits and confirms potential exposure to significant levels of this toxin from Maldive fish infected with fungi.

These results led to a new question: can fungal growth and mycotoxin production in Maldive fish be eliminated or reduced to safe levels? The most practical approach would be to reduce the a_w to sufficiently low levels that inhibit fungal growth and mycotoxin production. The limiting a_w levels for the most important species were therefore evaluated. The limiting a_w for growth of *A. tamarii* was between 0.82 and 0.85 on NaCl media and between 0.79 and 0.75, on media containing sugars at

ambient storage temperatures (25 to 35°C). The a_w of Maldive fish should be maintained below 0.75 to prevent the growth of *A. tamarii*. The physiology of *A. flavus* has been extensively studied previously but the limiting values are dependent on the food matrix. A smoked fish agar was used to simulate Maldive fish for fungal growth (*A. flavus*) and mycotoxin production (aflatoxin and CPA) under varying conditions. No growth occurred at an a_w of 0.75 while the toxin production was limited at an a_w 0.80 under all incubation conditions (25°C to 40°C). Hence, control of *A. flavus* can be achieved by rapid drying of Maldive fish to an a_w of 0.75 or below.

This study has provided scientific evidence that the mycoflora on Maldive fish produce aflatoxins and other mycotoxins that are a food safety risk. Hence, control of toxigenic fungi is imperative and can be achieved through adequate drying. This information is crucial for the Maldives as well as other developing countries that consume hot smoked dried fish while it potentially has a broader application for other food products.

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Contents

List of Abreviations

a_w	Water activity
AFPA	Aspergillus Flavus and Parasiticus Agar
CCA	Coconut Cream Agar
CPA	Cyclopiazonic Acid
CYA	Czapek Yeast Extract Agar
CY20S	Czapek Yeast Extract Agar with 20% Sucrose
DG18	Dichloran 18% Glycerol Agar
DRBC	Dichloran Rose Bengal Chloramphenicol Agar
G25N	25% Glycerol Nitrate Agar
HPLC	High Performance Liquid Chromatography
HPLC-FLD	High Performance Liquid Chromatography with Fluorescence Detection
MEA	Malt Extract Agar
MY5-12 and MY10-12	Malt Extract Yeast Extract 5% (or 10%) Salt 12% Glucose Agar
OTA	Ochratoxin A
SFA	Smoked Fish Agar
TLC	Thin Layer Chromatography
YES	Yeast Extract Sucrose Agar

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Mohamed, S., Mo, L., Flint, S. Palmer, J., and Fletcher, G.C. (2012). Effect of water activity and temperature on the germination and growth of *Aspergillus tamarii* isolated from "Maldive fish". *International Journal of Food Microbiology*, *160* (2), 119-123. DOI:10.1016/j.ijfoodmicro.2012.09.022.

Mohamed, S., Flint, S., Palmer, J., Fletcher, G.C., and Pitt, J.I. (In Press). A simple screening method for detection of citrinin using coconut cream agar. *Letters in Applied Microbiology*.

Mohamed, S., Flint, S., Palmer, J., and Fletcher, G.C. (Submitted). Fungal toxins in Maldive fish, a smoked dried tuna fish. *New Zealand Food Technology*.

List of Conference Proceedings

Mohamed, S., Mo, L., Flint, S. Palmer, J., Fletcher, G.C. and Pitt, J.I. (2011). Effect of water activity and temperature on the germination and growth of *Aspergillus tamarii* isolated from smoked dried fish. New Zealand Microbiological Society Annual Conference, 23-25 November, Palmerston North, New Zealand.

Mohamed, S., Flint, S. Palmer, J., Fletcher, G.C. and Pitt, J.I. (2012). Assessment of the mycotoxin producing potential of toxigenic fungi isolated from smoked dried fish. New Zealand Institute of Food Science and Technology Annual Conference, 26-28 June, Hamilton, New Zealand.

Mohamed, S., Flint, S. Palmer, J., Fletcher, G.C. and Pitt, J.I. (2013). Isolation and Identification of Mycoflora and Toxigenic Fungi from "Maldive Fish". New Zealand

Institute of Food Science and Technology Annual Conference, 2-4 July, Hawke's Bay, New Zealand. Submitted.