UNIVERSITY OF JOHANNESBURG

AN INVESTIGATION OF FUNGI AND MYCOTOXINS IN BARLEY GRAIN AND MATERIALS USED FOR BREWING

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AN INVESTIGATION OF FUNGI AND MYCOTOXINS IN BARLEY GRAIN AND MATERIALS USED IN BREWING

By

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DISSERTATION

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ABSTRACT

Mycotoxins, secondary metabolites of filamentous fungi, are associated with foods due to the ubiquitous nature of certain fungi that infect crops during harvesting or storage. These toxins have been implicated as chemical agents of acute and chronic diseases in animal and man. The most commonly acute effects of mycotoxin poisoning is the deterioration of the liver and kidney functions, allergic responses and immunosuppresion, whereas chronic effects include mutagenicity, teratogenicity and carcinogenicity. The most common toxigenic fungal genera include *Aspergillus*, *Fusarium* and *Penicillium*. Aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FBs), trichothecenes and zearalenone (ZEA) are the most important mycotoxins in terms of occurrence on food.

A study was conducted to evaluate and quantify the occurrence of mycotoxins in barley as well as barley-producing beer products in South Africa. A total of 86 barley samples were randomly obtained from Gauteng retail outlets, Maltsters and South African Breweries and were screened for toxigenic fungi. Two fungal genera, *Aspergillus*, *Penicillium* occurred regularly whereas *Fusarium* and *Mucor* were detected at low incidences. High levels of fungal contamination were found in barley obtained in Gauteng as compared to Maltsters barley samples, however, most of the fungal strains isolated from Gauteng purchased barley were non-toxigenic as compared to Maltsters.

Barley samples were further screened for mycotoxins by multi-mycotoxins extraction coupled with thin layer chromatography (TLC). Mycotoxins detected in the barley extracts were aflatoxins, ochratoxins, deoxynivalenol (DON) and zearalenone at trace levels on the thin layer chromatograms. However, TLC only indicated qualitative results. The presence of the toxins were confirmed by techniques that a highly sensitive and quantitative, such as gas chromatography-mass spectroscopy (GC-MS) and immunoaffinity analysis.

The presence of deoxynivalenol in the barley fractions was confirmed by GC-MS at mean concentration levels ranging from 0.0628 to 0.832 ppm. Barley samples from Maltsters, however, showed to be highly contaminated with DON compared to barley

obtained in Gauteng ($p < 0.05$). As barley is known to be one of the major ingredients of beer, a total of 48 beer samples were also randomly collected from retail outlets in the Gauteng region and were surveyed for the presence of AFB_1, AFB_2 and ochratoxin A. Trace levels of AFB_1 were detected in some of the beer samples, whereas AFB_2 was not detected. Ochratoxin A contamination, however, in beer ranged from 0.07 to 0.081 ppb.

The level of mycotoxins contamination in barley samples analysed by immunoaffinity analysis ranged from: 0.0 to 3.9 ppb AFs, 5.0 to 10.0 ppb OTA, 0.0 to 10.0 ppm DON, 0.0 to 5.0 ppm FBs and 0.4 to 2.9 ppm ZEA in Maltsters barley, whereas in Gauteng samples mycotoxin contamination levels ranged from 0.0 to 6.0 ppb OTA, 2.0 to 2.0 ppm DON, 0.0 to 2.0 ppm FBs and 0.5 to 3.4 ppm ZEA. Although high fungal infection was found in Gauteng samples, Maltsters samples were found to be more contaminated with mycotoxins ($p < 0.05$).

An investigation was also conducted to confirm the natural occurrence of fumonisin B_1 (FB_1) in barley samples at levels of up to 5 ppm, as determined by Vicam immunoaffinity analysis. The HPLC analysis was used to determine FB_1 in these barley samples. HPLC analysis of the barley samples previously found to be positive for fumonisins revealed detectable levels of ≤ 0.21 ppm FB_1 in only 7 samples of the 24 samples analysed.

Materials found to contain fungi and mycotoxins were further examined for cytotoxicity using human lymphocytes for possible chronic effects. Pure mycotoxins and selected barley fractions were found to be toxic to the lymphocytes. A study was also conducted to determine whether cytotoxicity testing could be used as additional tool for estimating the amount of toxin present in a commodity.

The differences in the level of fungal and mycotoxins contamination between Gauteng and Maltsters could have been due to the difference in the environmental conditions, which the barley was harvested, or the varying degree of handling and storage within the companies. This study may also present the general picture on the quality of products in the brewery industry. Although some of the barley samples were of low quality in regards

to food safety, the issue of upgrading quality control measures in the barley producing regions in South Africa will be of paramount importance.

DECLARATION

I hereby declare the dissertation, which I herewith submit for the research qualification

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to the University of Johannesburg is, apart from recognized assistance, my own work and has not previously been submitted by me to another institution to obtain a research diploma or degree.

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