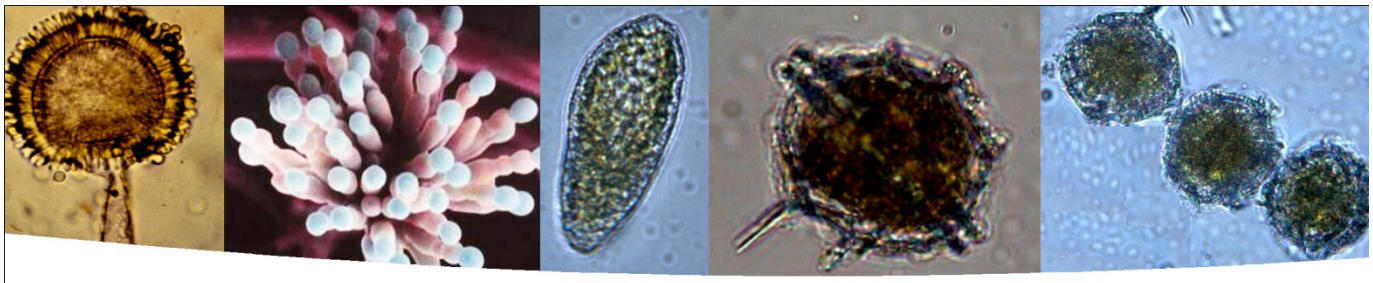

PROCEEDINGS

VI LATIN AMERICAN CONGRESS OF MYCOTOXICOLOGY
and
II INTERNATIONAL SYMPOSIUM
ON ALGAL AND FUNGAL TOXINS FOR INDUSTRY

June 27 to July 1, 2010

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09:30-10:00 AQUEOUS OZONE, A FRIENDLY METHOD FOR AFLATOXINS DEGRADATION

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Background: Aflatoxins (AF) are highly toxic and hepatocarcinogenic metabolic compounds produced by *Aspergillus* species such as *A. flavus*, *A. parasiticus* and *A. nomius* during infection and colonization of food raw materials as cereals, pulses and tree nuts and their by products. Several chemical methods have been shown to be effective on removing aflatoxins; however, they need to fulfill many characteristics to be approved by regulators agencies or, in some cases, they are still too expensive to be feasible on an industrial scale. One of the agents with great potential to reduce mycotoxins is ozone. It is effective against many mycotoxins and leaves no toxic residues after treatment.

Aim: to evaluate the use of aqueous ozone on degradation of AFB_1, AFB_2, AFG_1 and AFG_2.

Materials and Methods: Water saturated with ozone was prepared by bubbling gas, generated by passing ***extra-dry oxygen through*** an air-cooled corona discharge generator (Model CD-COM-HF-4) for 10 minutes (with the power generator at 100%; gas flow of 25 L / hours) in a bottle with 1000 mL of Milli-Q water, at 3 ºC. A concentrated ozone solution with *c.a.* 20 mg/L was obtained and was diluted with ozone demand free water. An aflatoxins stock solution (AFB_1 and AFG_1 – 2 mg/L each and AFB_2 and AFG_2 - 0.5 mg/L each) was used and it was diluted with ozone demand free water to the final working concentration. The degradation of mycotoxins by aqueous ozone was tested at 5 defined aqueous ozone levels: 0 (Control), 0.1, 1.0, 10 and 20 mg/L. A volume of 20 μL of each aflatoxin solution was applied into 2 mL vials, containing 200 μl of each aqueous ozone solution or ozone demand free water (Control) and left reacting for 30 minutes. To stop the reaction, 1200 μL of the mobile phase (see below) was added. Five replicates at each condition were made. Samples were analyzed using a HPLC equipped with a Jasco FP-920 fluorescence detector at 365 and 435 nm (excitation and emission, respectively), using photochemical post-column derivatization (PHRED unit). Chromatographic separation was performed on a reverse phase C_{18} column (Waters Spherisorb ODS2, 4.6 x 250 mm, 5 μm), fitted with a pre-column with the same stationary phase. The mobile phase used was pumped at 1.0 mL/min and consisted of an isocratic program as follows: water:acetonitrile:methanol (3:1:1, v/v). The injection volume was 100 μL.

Results and Discussion: AFB_1 and AFG_1 were more sensitive to ozone treatment than AFB_2 and AFG_2, since ozone acts preferentially against unsaturated compounds, by what may be classified by an electrophilic attack. The higher sensitivity of AFB_1 and AFG_1 is due to the 8,9 double bond forming the vinyl ether at the terminal furan ring (Mackenzie et al, 1998; Proctor et al, 2004), which is not present in AFB_2 and AFG_2. These results reinforce the relevance of this degradation strategy, since the more toxic aflatoxins (AFB_1 and AFG_1) (IARC, 2002; Proctor et al, 2004) are also the most sensitive ones to ozone. The highest degradation level for AFB_1 was 100%. The effective degradation of AFB_2, AFG_2 and AFG_1 was 8.06, 15.96 and 98.49%, respectively. As expected, the maximum degradation occurred at the highest ozone level (20 μg/mL).
Conclusion: Aqueous ozone can be used as a treatment for AF decontamination. Ozone foaming ability allows it to be trapped in a better way in water, since water is an industrial vehicle for washing and cleaning processes. However, gaseous ozone was found to be more effective then aqueous ozone, in the degradation of AFB_1 from dried figs (Zorlugenç et al., 2008).

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Acknowledgements: The authors thank Dr Scussel, V.M. from Food Science and Technology Department of the Federal University of Santa Catarina, SC, Brazil, for suggestions, comments and for Rocha, M.W. supervision during the Brazil-Portugal student exchange. Célia Soares was supported by a grant from Fundação para a Ciência e Tecnologia (reference SFRH/BD/37264/2007)