# Aflatoxin Detoxification Method Combining Mesoamerican Nixtamalization and Clay Absorption Techniques

An Undergraduate Research Scholars Thesis

By

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# ABSTRACT

Aflatoxin Detoxification Method Combining Mesoamerican Nixtamalization and Clay Adsorption Techniques. (May 2013)

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Aflatoxins are potent carcinogens produced by fungi *Aspergillus flavus* and *Aspergillis parasiticus*. Aflatoxins are able to contaminate different crops. Mexico has a pertinent climate that promotes production of aflatoxins in corn, which is a highly consumed crop. Once ingested in the body, aflatoxins are able to cause harmful effects such as liver cancer. Mesoamerican cultures have used the nixtamalization food process on maize since the Aztec and Mayan civilization periods. The process has proven to be effective in reducing aflatoxin concentration within maize. Extensive research has shown the effectiveness of clays, particularly the montmorillonite-rich bentonite clays, to adsorb aflatoxins. This research is aimed to combine both techniques to evaluate their synergistic effects on aflatoxin detoxification.

Montmorillonite samples that have previously shown high aflatoxin adsorption capacity was utilized. The aflatoxin adsorption capacity of the montmorillonite samples was evaluated using adsorption isotherms described in Kannewischer et al., (2006) with the modification of preparing aflatoxin solutions in lime saturated water (1.5 g/L $Ca(OH)_2$) with a very basic pH, in order to simulate the nixtamalization process. The concentration of aflatoxin left in the solution was analyzed using a UV-Visible Spectrometer.

After sampling the aflatoxin solution at different pH's varying from alkaline, to represent nixtamalization, to acidic, the experiments proved that there was reformation of aflatoxin within stomach pHs of 2 to 3. The aflatoxin changed chemical structure once exposed to alkaline pH, causing it to open its lactone ring structure and become more solubilized in water. Due to the change in chemical structure and conversion of the nonionic species to an anionic species, which was repelled by the negatively charged montmorilloinite, the adsorption of this new structure by the clay was minimal if any, while when in acidic conditions, the aflatoxin was adsorbed well by the montmorillonite. The preliminary results implied that combining the traditional Mexican nixtamalization food process and clay incorporation in processed corn products may further limit the bioavailability of aflatoxins compared to the individual methods along.

# DEDICATION

To my grandmother, Lucia Maldonado, for always believing in me and giving me unconditional love and support.

# ACKNOWLEDGEMENTS

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# CHAPTER I

# INTRODUCTION

Aflatoxins are toxic organic compounds produced by the fungi *Aspergillus flavus* and *A. Parasitcus*. They are examples of mycotoxins, which encompass a variety of toxins, mostly secondary metabolites that are known by their negative effects on animal and humans. Aflatoxins contaminate different crops comprising of corn, wheat, and rice. Aflatoxins are heat stable and are able to survive different food processes(Phillips et al., 2002). Their ability to survive numerous food processes is detrimental to the human population.

Countries located within the "hot zone" provide suitable ambient conditions for aflatoxin occurrence. Mexico is located in this zone, where the conditions of high temperature and drought increase the aflatoxin occurrence. The Mexican population has a high rate of corn consumption due to foods like tortillas. The concentrations of aflatoxins found in crops, in this case corn, are related to several health concerns, specifically focusing on liver cancer. It is essential to find a resourceful, economical, and efficient way to detoxify grains for the benefit of human and animal health.

Nixtamalization is a traditional Mesoamerican process for the preparation of maize, or corn, in which the grain is immersed and slowly cooked in an alkaline solution of limewater and then hulled. These processes not only have been able to improve the flavor, but have also proven to dramatically reduce the mycotoxin concentration by chemically inactivating the aflatoxins. Nixtamalization is not the only process that has proven to be able to reduce aflatoxin

availability. Since the 1970's, researchers have concluded that clay minerals are able to adsorb aflatoxins. The major clay mineral that reduces aflatoxin concentrations is montmorillonite. This is a 2:1 layer structure aluminosilicate mineral. Aflatoxins can be adsorbed at the edge surfaces as well as within the interlayer of the mineral. Research has shown that aflatoxin is adsorbed in the interlayer space of the montmorillonite(Deng et al., 2010). Furthermore, the adsorbed aflatoxin in smectite complex is highly stable to water washing, which suggests high affinity of the clay mineral for the toxin

**Objectives**

1) To conduct aflatoxin detoxification by combining two detoxification processes: nixtamalization and clay adsorption.
2) To evaluate the effectiveness of the combined detoxification method.

# CHAPTER II

# METHODS

A Texas bentonite (coded as 8TX) sample previously showed high aflatoxin adsorption capacity. The unfractionated sample was used to test the efficacy of the clay to adsorb aflatoxin in lime-saturated water. The aflatoxin adsorption capacity of the montmorillonite samples will be evaluated using adsorption isotherms described in Kannewischer et al., (2006) with the modification of preparing aflatoxin solutions in lime saturated water (1.5 g/L $Ca(OH)_2$) with a very basic pH of roughly 12.3, in order to simulate the nixtamalization process.

The concentration of aflatoxin left in the solution was analyzed using UV absorption. High Performance Liquid Chromatography (HPLC) was used to analyze the concentration if the lime saturated water absorbance interferes with the absorbance reading of the aflatoxin.

# CHAPTER III

# RESULTS AND ANALYSIS

Preliminary experiments were performed to determine a) whether cornmeal would suffice as a corn substitute and b) whether the UV spectrum can be used to quantify aflatoxin B1 in lime saturated water. Corn and corn meal extracts were obtained in DI water and Ca(OH)2.

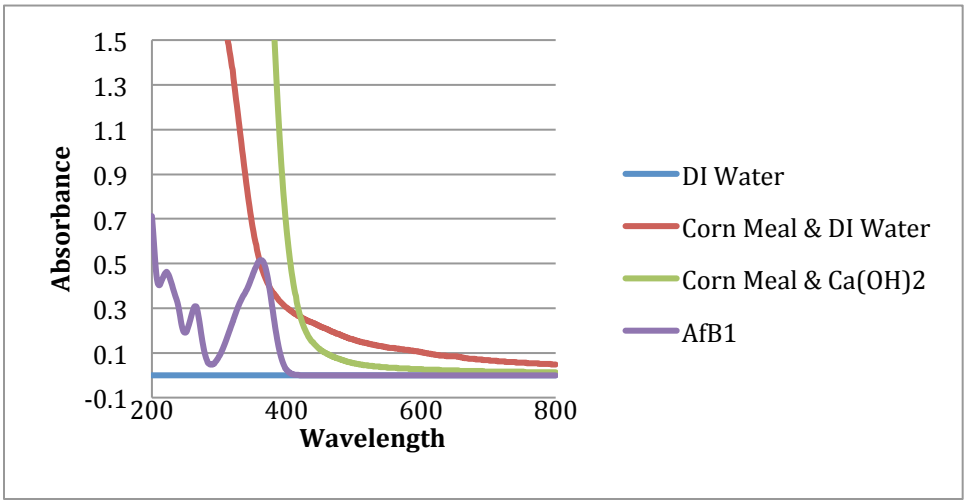


**Figure 1. The UV-Visible spectra of the supernatant of corn meal mixed with DI water and lime water. The spectra of DI water and AfB1 solution were plotted in the figure for comparison.**

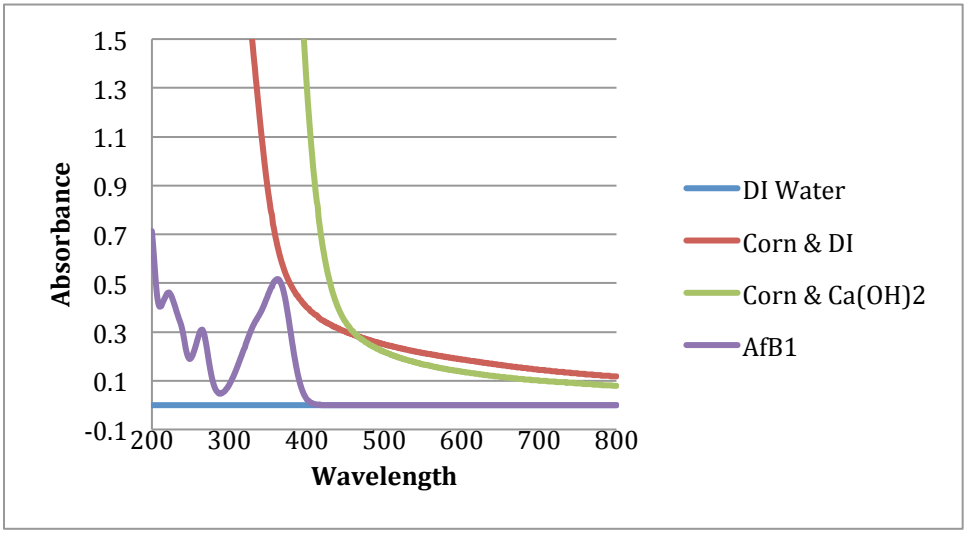


**Figure 2. The UV-Visible spectra of the supernatant of Corn mixed with DI water and lime water.**

Figure 1 and Figure 2 both demonstrate that the supernatant of the solutions from the corn and the corn meal in DI water and in lime water had similar spectra that interfere with the aflatoxin absorbance. Both the corn and the corn meal run off the graph in the area of interest, a different method has to be used to quantify the absorption of the aflatoxin in solutions that have contact with corn.

The pH of the aflatoxin in $Ca(OH)_2$ solution varied between 11 to 12, ultimately demonstrating a very basic solution that simulates the nixtamalization process. To demonstrate the changes in the aflatoxin molecule when subjected to different pH solutions, the saturated limewater was titrated with HCl to obtain solutions of pH 12 and 7-2. Additionally, clay was incorporated in the solutions to observe any adsorption of aflatoxin by the clays. Each treatment was duplicated to ensure minimal experimental error.

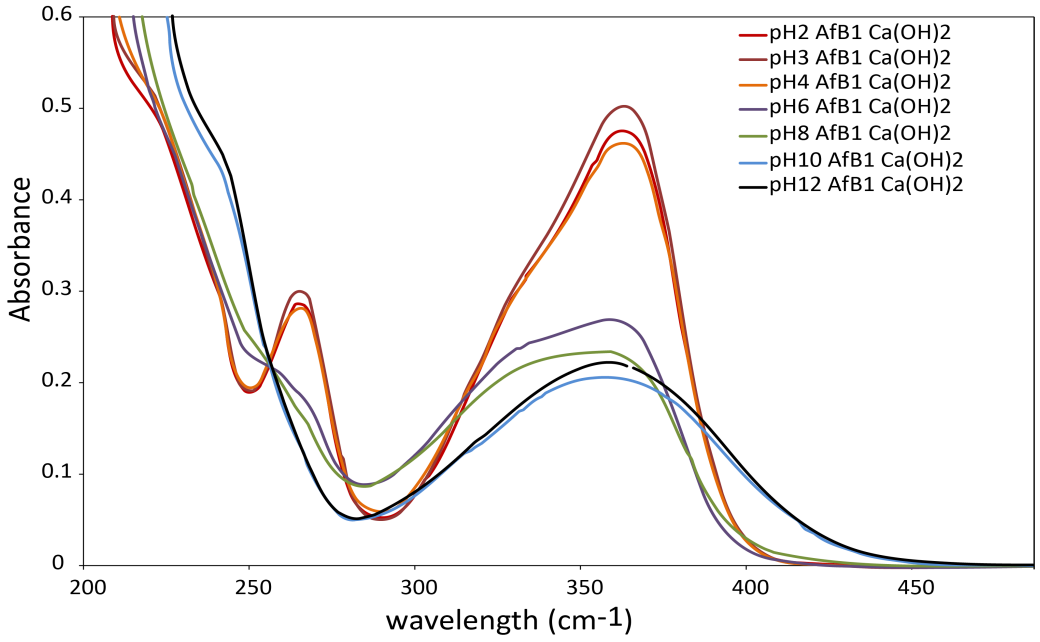


**Figure 3. The UV-Spectra of aflatoxin in lime water and acidified solutions in the absence of clay.**

The ideal aflatoxin B1 band is the one that is more potent and able to reach the absorption of 0.5 at wavelength of 362 cm-1. Figure 3 suggested that the chemical structure of the aflatoxin B1 was altered at high pH values, but was recovered by acidifying the solution.

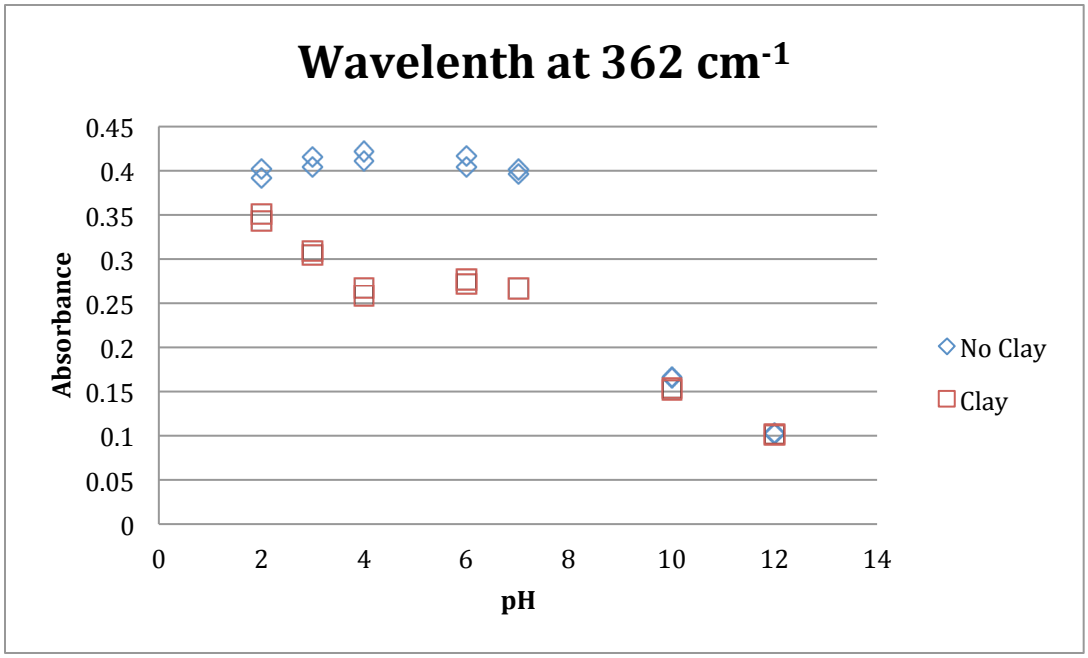


**Figure 4. Absorbance of aflatoxin solutions at wavelength 362 nm**

Figure 4 shows the absorbance of 8ppm AfB1 at the wavelength 362 nm and the different pHs, again, both with and without clay. The difference in absorbance between the solutions with clay and the solutions without clay suggest that aflatoxin adsorption took place in all of the acidic solutions, with the greatest adsorption at pH 4. At the basic solutions, however, little to no adsorption took place, as demonstrated by pH 12.

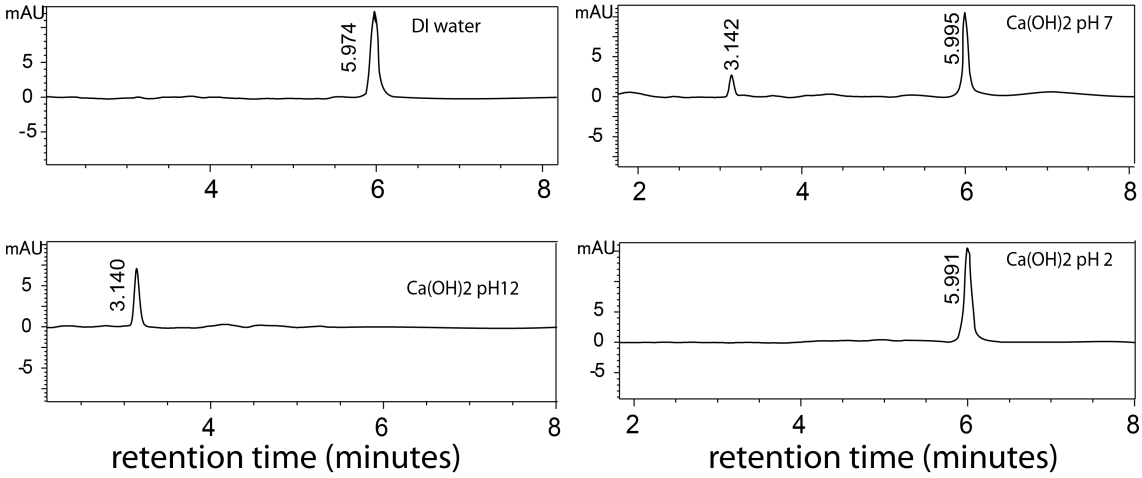


**Figure 5. HPLC chromatographs of AfB1 at different pH**

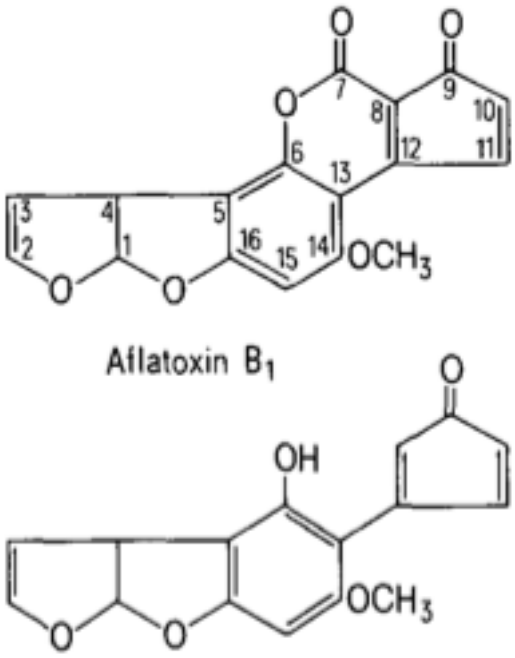


**Figure 6. Aflatoxin B1 chemical structure, top, and structural change of molecule after subjected to alkaline solution, bottom (Figure from Lee et. al 1980.)**

The HPLC results in Figure 5 confirm that the aflatoxin B1 molecule undergoes changes in the alkaline pH produced by the limewater. The retention time of AfB1 in DI water was observed at 5.9 minutes. This retention time shifted to 3.1 minutes at a high pH. The further acidification of the solution caused the retention time to go back to 5.9 minutes. Figure 6 demonstrates the chemical structure of the aflatoxin B1 molecule. Once the aflatoxin molecule was subjected to

the alkaline nature of the lime water solution, the lactone ring, represented by the carbon groups 6, 7, 8, and 13, experienced hydrolysis. This ultimately causes the temporary loss of one of the oxygens until the solution is acidified, with that, the recovery of the AfB1 molecular structure.

# CHAPTER IV

# CONCLUSION

The aflatoxin B1 changed chemical structure once exposed to an alkaline pH. The basic solution caused hydrolysis of one of the lactone rings in the molecular structure to open; therefore it became more solubilized in water. This chemical structural change converted the nonionic species to an anionic species. The new anionic species was repelled by the negatively charged montmorillonite, in contrary to the significant adsorbance of the aflatoxin by the montmorillointe in acidic conditions. There was a marginal adsorption, if any, of the new aflatoxin structure by the clay.

Based on the data from the experiments, combining the Mesoamerican Nixtamalization food process and the clay adsorption methods at high pH did not prove to remove more aflatoxin. The clay can adsorb aflatoxins after acidifying the nixtamalized aflatoxin simulation solution. The experiments from the Nixtamalization simulation proved the removal of aflatoxin in the process was mainly solubilizing aflatoxin. There is reformation of the aflatoxin B1 strain at low pH. Adsorption of aflatoxin on the clay at the low pH can occur and therefore, may further reduce the toxicity of aflatoxin residue or reformed aflatoxin after the nixtamalization.

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