Nixtamalization as a Method to Reduce Aflatoxin and Increase Available Nutrients in Maize

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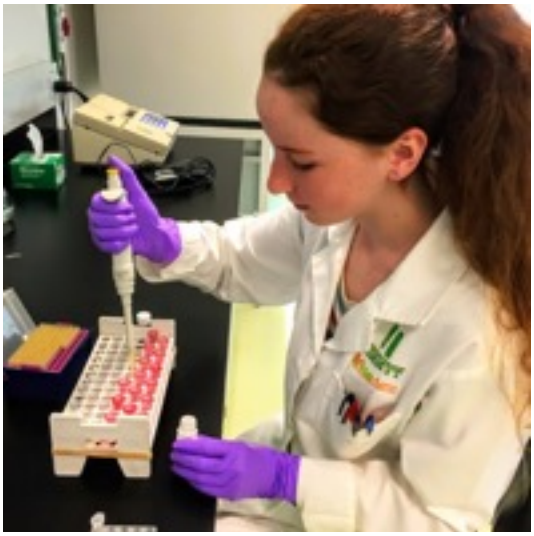

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ACKNOWLEGEMENTS

This experience was truly inspiring, but it did not come without its challenges. The most difficult being lack of experience in working in a lab and in the topics that were being researched. I have never worked in a lab setting before, so it took some adjusting to understand the reasoning and methods behind daily tasks. This required asking questions and asking for help when I needed it. The purpose of my time at CIMMYT was to learn, and part of that was learning how to manage tasks and take control of my success. Another challenge that came with living in a different country was the language barrier. I knew very basic Spanish, but not to the level necessary to understand technical scientific instruction or analysis. Thankfully my research mentors spoke some English, but the majority of the researchers only spoke Spanish. This made it difficult to understand some of the complex procedures and analyses we were conducting, but it also gave me the opportunity to learn Spanish and how to communicate without solely relying on words. Things did not always go as planned in the lab during my research, so I had to problem solve and work with others to find or create a solution. The equipment that was the primary data collection device was malfunctioning and did not produce consistent results; Without this piece of equipment, the experiment could not be completed. Solving this issue took creative thinking, patience, and understanding that it was acceptable to face challenges. This realization came at a very opportune time because I faced many more interruptions. One of the biggest logistical challenges I experienced was the window of delivery for the maize samples I

would be analyzing. They were delivered very late in my internship, so I did not have the opportunity to finish all the data collecting myself. Fortunately, I was not alone in the research and the Maize Nutritional Quality team assisted me in every step including finishing collecting data. My time in Mexico was a learning experience in every way, I gained new skills in problem-solving, time management, organization, and gained a whole new worldview.

First and foremost, I would like to thank the late **Dr. Norman Borlaug** for sharing his passion and commitment to solving the global hunger crisis by creating the World Food Prize Foundation. This organization is a champion in showcasing what young people can do to create change. I am forever grateful to have had the opportunity to be a Borlaug-Ruan International Intern and carry on the legacy of Dr. Norman Borlaug. I would also like to extend my gratitude to the **Ruan family** and **Ambassador Kenneth M. Quinn** for recognizing the potential of young people as leaders in the fight against food insecurity.

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ABSTRACT

Aflatoxin, a form of mycotoxin, is a secondary metabolite found in soil and organic matter. They are produced by microfungi and have very detrimental effects on human and animal health as well as food security. Contamination by aflatoxin is largely inevitable in crop production, especially in drought conditions and intermediate temperatures. Aflatoxin are also difficult to eliminate during food processing because of their resistance to heat, chemical and physical treatments, and other widely used food processing methods. The most common strains are *Aspergillus flavus* and *Aspergillus parasiticus* which produce four aflatoxin types: B_1, B_2, G_1, and G_2, that are distinguished by the blue or green fluorescence under UV light. These strains of aflatoxin are the most potent natural carcinogen. B_1 produced by *A. flavus* will be referred to as "aflatoxin" because it is the only strain present in the data. The versatility and ease of maize, as well as the rich nutritional profile, makes it a go-to for many families in low-income areas, especially where food insecurity and malnutrition are present. However, when contaminated by aflatoxin, the nutrients in maize are quickly degraded. Nixtamalization as a processing method for maize reduces the risk of disease by contamination and provides many improved nutrition benefits to target the issue of food insecurity. This study aimed to determine if nixtamalization was a practical and efficient method to reduce aflatoxin and increase nutrients in maize native to Mexico. The results show that after nixtamalization, maize samples had considerably less contamination than before. More research into nixtamalization as a process to improve the nutrition of maize and reduce the presence of aflatoxin is important in the fight against malnutrition and food insecurity in a growing world.

KEY WORDS: Maize, Aflatoxin, Nixtamalization

INTRODUCTION

Mycotoxins are secondary metabolites produced by microfungi that have very detrimental effects on human and animal health. Contamination by mycotoxins is a global concern because of its unpredictable and unavoidable nature even with good processing and storage practices. This paper will specifically discuss aflatoxins, a form of mycotoxin, and their role in plant pathology. Aflatoxins are mainly found in soil and organic matter, which puts crops at a high risk of contamination and therefore livestock and humans who consume the crops. Health effects of aflatoxin are targeted in the liver, but they are also mutagenic, carcinogenic, teratogenic, hepatotoxic, and have immunosuppressive effects (Bennett & Klich, 2003).

The four major aflatoxins are B_1, B_2, G_1, and G_2, they are distinguished based on the color of their fluorescence under UV light, either blue or green. There are many strains of fungi that produce aflatoxin, but the most common are *Aspergillus flavus and Aspergillus parasiticus*. *A. flavus* is capable of producing only B_1, B_2, but *A. parasiticus* can produce all four types. These specific strains of aflatoxin are considered to be the most potent natural carcinogen (Squire, 1981). B_1 produced by *A. flavus* is the most common and broadly known aflatoxin, because of this, the term “aflatoxin" will be used to refer to the B_1 strand produced by *A. flavus* for the remainder of this paper.

Aflatoxin contamination is highly variable in plants. Natural contamination occurs commonly in cereal grains such as corn, soybeans, wheat, peanuts, and many other foods. This contamination can occur before harvest or after harvest while in storage. Contamination before harvest has been associated with drought conditions, and other factors that weaken plant structure. The contamination after harvest is attributed to the poor storage environments of

harvested crops that supply optimal conditions for mold and aflatoxin growth such as high moisture content and humidity. The methods to prevent uncontrollable aflatoxin growth are mostly preventative. This can include proper agricultural practices before, during, and after the growing period, as well as drying of the crops after harvest to ensure the moisture content is low enough to inhibit mold growth. Even with good practices, aflatoxin contamination is largely inevitable and not easily eliminated during food processing. Aflatoxin are very resistant to heat, chemical and physical treatments, and other widely used food processing methods (Alshannaq & Yu, 2017).

Specifically discussing the aflatoxin plight in maize crops, areas with ambient temperatures and dry conditions are most affected, such as the southern United States, Mexico, India, and Africa. Aflatoxin can be easily identified with just the human eye by a yellow-green or grey mold on the kernels, husks, and/or stems of maize crops. Along with being easily identifiable, aflatoxin are easily spread. Aflatoxin are a saprophytic organism (obtaining energy from dead and decaying organic matter) that spread by asexual spores the fungi produces. These spores can be carried by the wind or insects and enter through any point on the crop that is structurally compromised. Insects, including the European corn borer, sap beetle, and corn earworm, can also provide direct entry of the spores into the plants. (Richard, 2007). Contamination of grains used in animal feed can be carried through to animal products humans eat such as eggs, milk, and meat. This presents a very difficult situation for developing nations related to human health and food insecurity, but it also affects developed nations. There have been proposed solutions for controlling the toxin manifestation in food and crops, however, like the issue of food insecurity, no single solution exists. As stated by Brown et al. (1998), recent

advances in research of plant breeding and gene editing/engineering have created the opportunity to develop more fungi-resistant crops. This coupled with targeting genes engaged in regulatory processes of mycotoxin development and the use of biocontrol agents have given an advantage to farmers. Other postharvest prevention methods include detection and isolation of aflatoxin contaminated food by government regulation and screening programs (Bennett & Klich, 2003). However, because aflatoxin is a natural contaminant, none of these solutions have solved the problem.

Role of Maize in Nutrition and Food Security

Maize, also referred to as "corn", from a nutritional view, is an excellent source of proteins, starch, lipids, and other nutrients. This crop is the major food source for one third of the world's population. Many undeveloped and developed nations such as Mexico, Africa, Southeast Asia, Latin America, and parts of the United States, rely on maize as a primary food (Nuss & Tanumihardjo, 2010). The versatility and ease of maize and its derived products makes it a go-to for many families in low-income areas, especially where food insecurity is present. The anatomy of a corn kernel has four primary structures, the pericarp, germ, endosperm, and tip cap. The endosperm makes up 83% of the kernel and is where a majority of the starch is found with a small amount of protein and vitamins and minerals. The germ is a large storage of fiber, protein, iron, and zinc; and the pericarp and tip cap are the outer protective shields of the kernel. The pericarp, germ, and tip cap make up 5%, 11%, and 1% of the corn kernel, respectively. (Vanara, et al., 2018). This translates to about 72% starch, 10% protein, and 4% lipid (Inglett,1970). Maize supplies an energy density of about 3.65 kcal/1 gram which is similar to that of other life

sustaining crops; wheat yields 3.40 kcal/1 gram and rice yields 3.60 kcal/1 gram (USDA). However, the nutritional quality of these staple crops can be significantly reduced with the presence of mycotoxins, like aflatoxin.

Maize alone supplies an abundance of macro- and micronutrients, but the qualities of some of these nutrients are disproportionate and deficient for human metabolic needs. Maize alone cannot be relied on as a single source of food because it will lead to malnourishment. This is becoming a large issue in underdeveloped, poor, and rural areas where maize is the only source of consistent food that may be available. Some of these nutrients that are lacking in maize are essential amino acids lysine and tryptophan, vitamins C, B and A, iodine, and iron. Research in exogenous and endogenous maize fortification is being conducted to help improve macro- and micronutrient quality and quality to combat malnourishment. This encompasses biofortification of maize as well as making nutrients more bioavailable for absorption once consumed (Nuss & Tanumihardjo, 2010). Different geographic regions require different fortification. For example, provitamin A is most necessary in Zambia, Zimbabwe, Malawi, Colombia, Pakistan, Ethiopia, Nigeria, Democratic Republic of the Congo, and Mali. Increased protein quality, with respect to the deficient amino acids, is necessary everywhere in the world, but especially in South Africa; and high kernel-zinc maize is urgent in Guatemala, Nicaragua, and Colombia (Rojas, et al., 2016). Any degradation of the available nutrients by aflatoxin is detrimental to the communities that rely on maize as a majority of their daily calories.

Globally, government aflatoxin regulation varies between countries. The regulated maximum for aflatoxin by the United States Food and Drug Administration is 20 parts per billion

(ppb) for humans and animals. In the European Union, the maximum is much lower, around 0.1-15ppb depending on the food being produced. In Mexico, the regulated value for aflatoxin is 30 ppb, but the actual value in various food products tests much higher values (USDA, 2009).

Maize can be consumed and prepared in a multitude of ways that vary around the world: straight off the cob, boiled, ground, roasted, as animal feed, fermented, used in cakes, breads, and alcoholic beverages, and nixtamalized. Further processing can also yield sweeteners, oils, thickeners, and other by-products (Inglett,1970). As Nuss and Tanumihardjo (2010) state, "Maize is a dietary staple for more than 200 million people. This number can be expected to grow as the world's population approaches 8 billion in 2025, indicating maize's status as a paramount crop in the context of global nutrition." The importance of maize will only increase as the number of people needing nutritious foods increases, so research into the aflatoxin and nutrient fortification are necessary to feed the growing world.

Climate change is changing how the agriculture industry functions worldwide. Shifts in temperature, weather patterns, and other factors are creating and destroying growing areas resulting in loss of efficiency, crop yield, and income (Stepman, 2018). This change also increases the likelihood of pathogenic activity that could dramatically affect the economic and social systems in the agriculture industry (Magan, et al.) (Battilani, 2016).

Nixtamalization

Nixtamalization is a processing method for maize to increase nutritional value, decrease aflatoxin levels, and prepare the maize for food products. In a traditional nixtamalization process, maize is cooked and steeped in a solution of lime (calcium hydroxide) and wet-milled to

produce masa. The lime concentration usually contains 0.5-3% of calcium hydroxide mixed with water to create an over saturated solution. Part of the nixtamalization process requires washing and cooking of the maize. The maize is added to the correct solubility of lime solution (depending on the amount of maize), cooked in an open vat, and then left to steep overnight. The maize is then washed and the pericarp, which is where most of the aflatoxin reside, is manually removed from the kernels. The name 'Nejayote' is given to the liquid after cooking that contains pericarp fragments, remaining lime, and water. During this process, the maize kernels swell to about 1.5 times their original size; 28-30% of water absorption is done during cooking, and 5-8% during steeping. The amount of water absorbed is dependent on the kernel size, pericarp thickness, lime concentration, and the type of endosperm (McDonough, et al., 2001). Nixtamalization provides many improved nutrition benefits that can reduce the malnourishment issue when maize is the primary food in a diet. These nutrition and health benefits include: decreasing the risk of the disease pellagra by increasing niacin bioavailability, increasing calcium levels from absorption of the kernels during steeping with the calcium hydroxide solution, increasing resistant starch which supplies dietary fiber; and substantially decreases mycotoxin levels. Along with health benefits, nixtamalization also lengthens the shelf life of food products and creates businesses and market opportunities for income in a community (Rojas, et al., 2016). This process yields a dough called masa which is used in over 300 food products in Mexico, the most recognized being tortillas (Moctezuma-Zárate, 2015). More research into nixtamalization as a process to improve the nutrition of maize and reduce the presence of aflatoxin is important in the fight against malnutrition and food insecurity in a growing world. This study aimed to determine if nixtamalization is a practical food safety processing method for maize in the

household to industry setting; as well as how efficient nixtamalization is at reducing aflatoxin. Given the background into the anatomy of a maize kernel and aflatoxin, nixtamalization has great potential to decrease aflatoxin in maize varieties native to Mexico while increasing available nutrients.

METHODS

I. Participants

Enrolled in this experiment was maize from the southern and central regions in Mexico. Each sample of grain was treated with a chemical control agent, but that was not a relevant variable to this study. The color of the grain also varied from white to yellow, however this was also not a relevant variable. The color of maize is mostly based on preference for consumers. The maize samples were only analyzed for aflatoxin levels.

II. Apparatus and Materials

To collect the data in this experiment, the devices used were designed specifically for use in quantifying aflatoxin. A transportable box with a ultraviolet light was used to identify if there was a presence of aflatoxin in the grain sample. This can only be used to identify which samples were contaminated by aflatoxin, but a chemical analysis of the grain is needed to quantify the amount present. An analytical balance scale was also used to weight samples of grain for analysis. The precision and accuracy of this device was necessary for the detail and accuracy of this study. A cold storage room was used to store the samples of grain at -15°C to inhibit the growth of aflatoxin. This was necessary to ensure the amount of aflatoxin present on the grain samples was from the growing or harvesting period, not from storage and analysis in the lab.

Various millers were also used to grind the maize samples to different sizes. In order to quantify the aflatoxin with the equipment available the grain needed to be ground to at least 1.5 millimeters in diameter. The final apparatus was AccuScan. This equipment consisted of small strips, similar to pH strips, that absorbed aflatoxin in a sample and measured it in a handheld device. This equipment was the primary apparatus used to collect data for this study.

III. Procedure

To begin this experiment, each sample of corn was assigned a sample number; the numbers were assigned randomly based on the position of the sample bag in the pallet. The numbers ranged from 4854 to 4949 as a continuation of the numbers from past experiments in the lab. The purpose of the number was to easily track the samples during experiments to connect with background data in analysis. Once numbered, each sample was tested under ultraviolet light to check for the presence of aflatoxin. The sample was mixed to homogenize it and poured onto a tray so the bottom was fully covered. The tray was placed in a portable UV box and the number of grains that glowed due to aflatoxin contamination were counted and recorded; three trials were performed for each sample. To prepare for the preliminary chemical analysis of the grain, the samples were shaken to homogenize the maize and 500 grams of grain from each sample were weighed using an analytical balance scale. These samples were milled to produce flour at 0.5 millimeter and 1.5 millimeter thickness for each sample. The different thicknesses was an independent variable to determine if aflatoxin levels tested differently. 20 grams of the milled flour from each sample was weighed using an analytical balance scale and added to sample bottle labeled with the appropriate sample number. To this, one packet of MAX

1-G50 aqueous extraction was added from the AccuScan kit as a precursor for analysis. To create a solution, 50 millimeters of distilled water was added to each sample bottle with the maize flour and placed in a vertical sample rocker to vigorously shake the samples for three minutes. The consistent amount of flour and water measured for each sample analysis was a controlled variable. Once the flour was completely mixed with the water, the samples were left to rest for five minutes to separate and form distinct layers based on particle size; Only the top layer (smallest particle size) is needed for this analysis. The solution was carefully poured, so only the top layer is transferred, into the top of a syringe with a cotton ball at the base for filtration, and filtered into a labeled test-tube. 100 microliters of sample diluent (provided by the AccuScan kit) was pipetted into red sample cups. Before beginning analysis, the handheld AccuScan Gold reader (provided by AccuScan kit) needed to be programed to the "Mycotoxin Q+ MAX" category and specifically the "Afla Q+ MAX" test to analyze aflatoxin level. Once the reader was programed, 100 microliters of the sample extract was placed in a red sample cup (also labeled with the correct sample number) with the sample diluent and mixed five times using the pipette. After mixing, one "Reveal Q+ MAX for Aflatoxin" test strip was placed in the sample cup, so the end of the strip came in contact with the solution and could begin to wick, for six minutes. After the allotted development time, the strip was removed and placed into the AccuScan Gold reader to quantify the aflatoxins present. This procedure relied on following the procedure correctly and without error to ensure the correct results were obtained. This analysis was repeated for each sample (4854 to 4949). The dependent variable was the aflatoxin level, measured in parts per billion (ppb) for each sample. If the aflatoxin result was higher than 50ppb, the AccuScan could not provide an accurate reading, so the sample extract needed to be

diluted. A dilution by six times was the first dilution and was made using 500 microliters of distilled water and 100 microliters of the sample extract. The chemical analysis was preformed again and if the result was higher than 50ppb, an extraction by seven times was made and tested; this continued until the result was under 50ppb to ensure a correct quantification of aflatoxin in the sample. To counteract the dilution in the results, the aflatoxin number was multiplied by the amount it was diluted by; for example, a result of a dilution by six times was multiplied by six.

Nixtamalization and Tortilla Making:

To begin the nixtamalization process, un-milled maize grain was added to a cooking pot. The weight of each sample varied based on how much total sample was available, but the average weight was around 600 grams. The calculations of distilled water and lime (calcium hydroxide) were dependent on the initial weight of the grain sample. The amount of distilled water added to a sample (in milliliters) was double the weight of the sample. The amount of lime added was dependent on what solubility or percent concentration was necessary, for this trial 1% was needed, so the weight of 1% of the grain sample was measured in lime. The solution formed was alkaline with a pH of around 11. The pots with samples were then cooked for 35 minutes at a constant temperature of 80°C and then left to steep (off the heat) for around 16 hours. After steeping, the grain was strained and the Nejayote (liquid from straining) was collected and labeled with the grain sample number for chemical analysis of aflatoxin. The grain, now called nixtamal, was rinsed twice and during the first rinse, the pericarp on the grain was manually removed by rubbing the kernels and creating a disturbance. The “clean” grain was then put into a wet-miller and ground with water to create masa. The control variables were the percentage of

lime, proportions of reactants, time for cooking and steeping, and temperature for cooking. This process was repeated for each sample.

The masa produced from nixtamalization was then used to make tortillas. The masa is kneaded with water to the correct consistency needed for traditional tortilla making. 20 grams of the masa dough was weighed using an analytical balance scale and flattened using a traditional tortilla press to achieve the correct shape and thickness needed for cooking. The fattened dough was then placed onto a hot tortilla pan (on a stove) and cooked for approximately 17 seconds on each side. This process was repeated 20 times for each sample. Once the tortillas were made, they were shredded into smaller pieces (to create more surface area) and placed into a flash freezer to preserve the tortilla. Once completely frozen, the tortilla pieces were placed into a freeze drier to rid of any moisture. After frozen, the tortilla pieces were milled to both 0.5mm and 1.5mm thickness. The chemical analysis described above was repeated for the nixtamalized samples after adjusting the moisture level. Moisture was adjusted by adding water to the milled tortilla and mixing; the moisture after this process was between 10-12%.

RESULTS

This study aimed to determine if nixtamalization could be used as a food safety processing method to improve the nutrition of maize and reduce the presence of aflatoxin. This research is important in the fight against malnutrition and food insecurity in a growing world. Given the background into the anatomy of a maize kernel and aflatoxin, nixtamalization has the potential to serve as a life changing practice in food sustainability and human health. The primary measurement was in aflatoxin levels of maize samples from Mexico. Quantification of

aflatoxin was determined at each stage of nixtamalization; the grain, nixtamal, nejayote, masa, and tortillas; and compared to determine how efficient nixtamalization is as a methods to improve food safety. Each sample was nixtamalized at 1% and 1.5% lime (calcium hydroxide) concentration to test whether this variable had an effect on aflatoxin levels. The output of masa from nixtamalization was further cooked as a tortilla and the tortilla was also analyzed for aflatoxin levels. The changes in heat and pH during nixtamalization and cooking were of interest to observe how it effects aflatoxin.

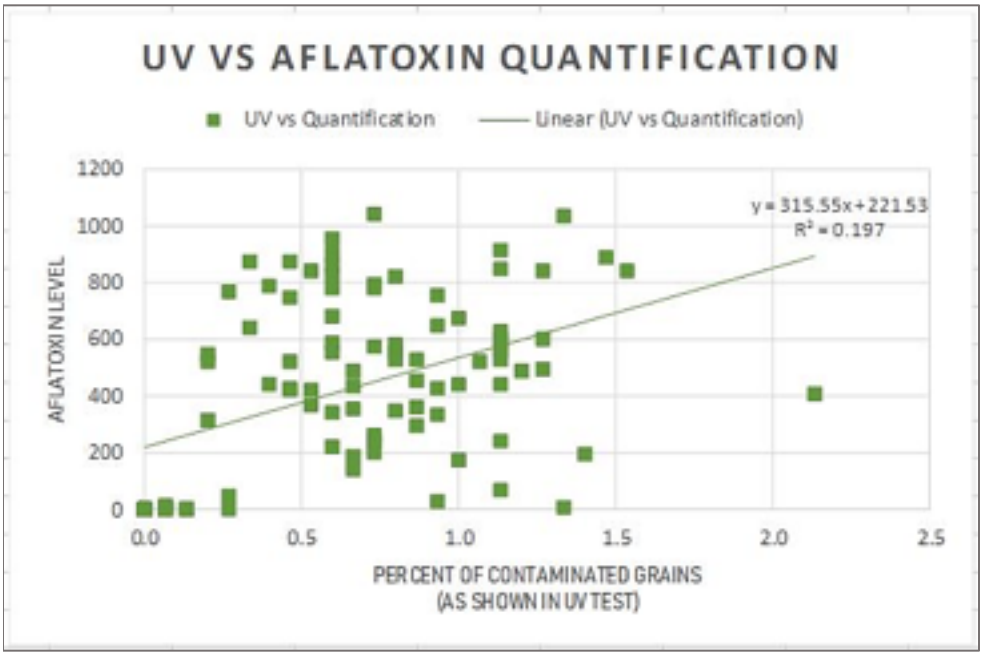


Figure 1. compares the percent of average grains contaminated found in the UV light test to aflatoxin quantification values found by performing chemical analysis.

The ultraviolet light analysis results of the grain samples show that there is a relationship between the amount of fluorescence and level of aflatoxin. While the test did not provide results on the qualification of aflatoxin, it was able to accurately predict the aflatoxin range based on percentage of grain contaminated. The samples with a similar percent of average grains that

Table 1. Displays the values from aflatoxin qualification of the outputs from nixtamalization: including raw grain, nixtamal, nejayote, masa, and tortillas.

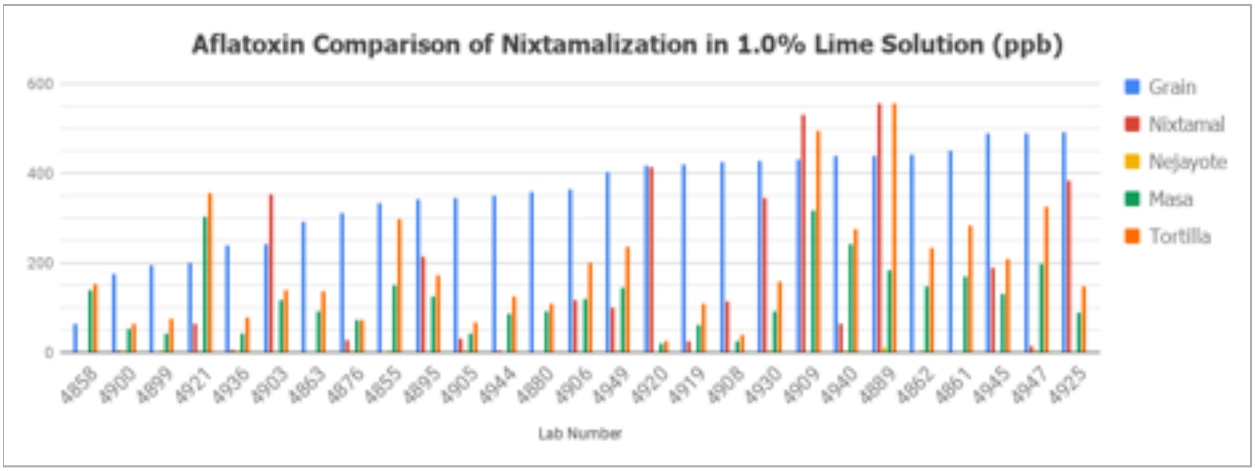
showed fluorescence had similar values when the chemical analysis for aflatoxin was performed

Lime Concentration 1%						Lime Concentration 1.5%					
Aflatoxin (ppb)											
Lab Number	Grain	Nixtamal	Nejayote	Masa-Tortilla	Tortilla	Lab Number	Grain	Nixtamal	Nejayote	Masa-Tortilla	Tortilla
Sinaloa cooked with water (no lime)	3	1.8	3.5	4.9	0.57	Sinaloa	3	1.7	0.2	4.3	2.4
Sinaloa	3	1.8	1.6	2.9	2.2	4858	66.0	175.5	1.8	6.3	231.6
4858	66.0	na	1.9	138.7	154.2	4900	175.2	4.1	0.3	25.8	42
4900	175.2	3.2	3	54.1	65.4	4899	194.4	3.3	0.0	32.6	49.2
4899	194.4	1.5	2.8	42.6	76.8	4921	202.2	739.2	1.3	114.6	202.2
4921	202.2	65.4	3	303.0	355.8	4936	238.8	7.6	0.7	34.1	189.6
4936	238.8	6.9	2.5	42.0	78.6	4903	243.0	468	0.2	112.2	268.2
4903	243.0	354.6	1.8	117	141	4863	293.4	8.9	1.9	112.2	113.4
4863	293.4	na	1.5	92.2	138	4876	311.4	495.6	3.4	92.4	123.6
4876	311.4	28.7	3.7	72	73.2	4855	333.0	18.2	0.2	322.2	301.2
4855	333.0	na	2.4	149.9	297.6	4895	342.0	6.5	0.0	99.6	254.4
4895	342.0	214.8	1.8	127.1	173.4	4905	346.8	9.1	2.6	90.6	175.2
4905	346.8	31	2.4	42.6	67.8	4944	351.0	13	2.0	78	195.6
4944	351.0	3.9	1.9	87.0	127.2	4880	360.0	18.9	2.9	283.8	256.2
4880	360.0	na	1.7	92.7	110.4	4906	365.4	2.1	3.0	29.6	69
4906	365.4	117	1.4	121.2	201.6	4949	404.4	2.8	0.9	127.2	224.4
4949	404.4	102	2.7	145.2	237	4920	417.6	5.2	2.9	81.6	61.8
4920	417.6	414	0.28	19.2	25.8	4919	421.8	276.6	0.6	40.6	62.4
4919	421.8	25.5	0.84	61.2	108	4908	427.2	33.9	0.3	29.6	65.4
4908	427.2	113.4		25.2	38.3	4930	429.0	1.8	0.5	93	183.6
4930	429.0	346.2	2.3	93.0	160.2	4909	431.4	3.8	2.8	133.8	259.2
4909	431.4	531	2	316.8	494.4	4940	438.6	1.6	0.6	19.4	61.8
4940	438.6	66	2.4	243.0	274.8	4889	439.8	30.1	3.1	432	411
4889	439.8	557.4	13	184.4	555.6	4862	442.8	281.4	2.9	34.3	46.2
4862	442.8	na	2.3	148.5	235.2	4861	450.6	26.2	2.4	114	124.8
4861	450.6	na	1.4	170	283.2	4945	489.0	445.8	3.1	64.2	199.2
4945	489.0	190.8	2	132.6	208.2	4947	489.0	6.1	1.0	64	175.2
4947	489.0	14.9	2.6	198.0	327	4925	492.0	157.8	0.7	85.2	243.6
4925	492.0	383.4	1.6	90.0	147						

The results of the nixtamalization section of this study show that overall the process of nixtamalization dramatically reduces aflatoxin levels in the maize samples that were tested. The values show that some of the aflatoxin was distributed to the najayote in the form of pericarp from the maize. It also shows that the cooking process from raw grain to nixtamal and from masa to tortilla had very little effect on aflatoxin level; this was expected because aflatoxin are very resistant to heat. When discussing the results of the two different lime concentration, both concentration effectively reduced aflatoxin in the maize samples, but 1.5% had a higher

Figures 2 & 3. Display the aflatoxin values from the outputs of nixtamalization in graphical form for ease of analysis.

reduction in aflatoxin than 1%. The higher lime concentration caused more aflatoxin to be transferred to the najayote which resulted in lower aflatoxin in the nixtamal.



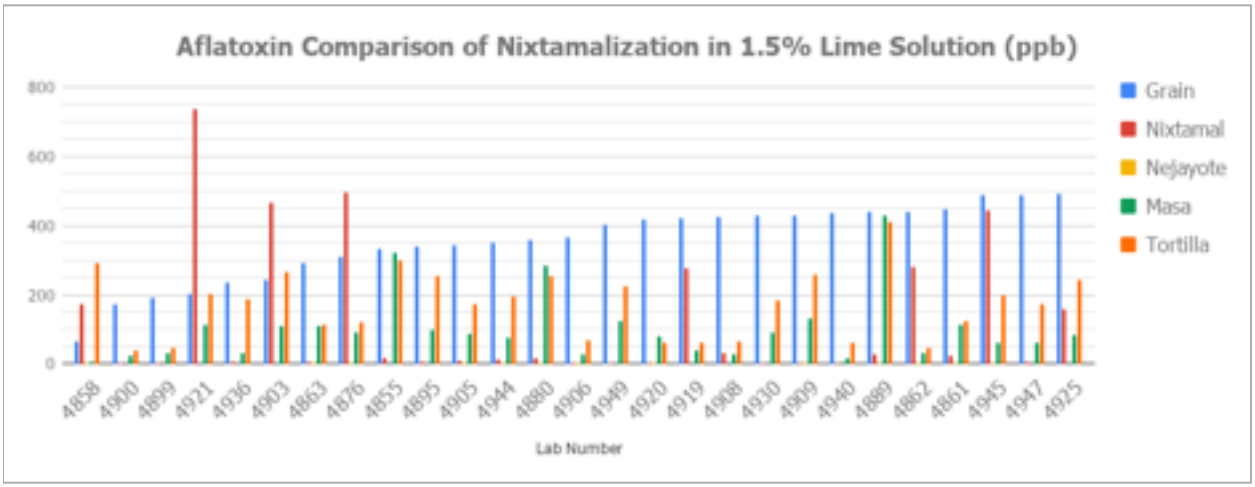


Figure 2 & 3. Displays the values from aflatoxin qualification of the outputs from nixtamalization: including raw grain, nixtamal, nejayote, masa, and tortillas in a graphical form for ease of analysis.

DISCUSSION

From the results of this study, it can be concluded that nixtamalization is an effective method to reduce aflatoxin in maize. Nixtamalization also increases nutrient availability to make maize a more nutritiously well-rounded staple crop. This conclusion is important as a possible solution to malnutrition and food instability in low-income areas. Maize is heavily relied on for a majority of daily nutrients and calories, so determining a way to make this crop have a larger impact would have beneficial effects on countless populations of people. The large reduction in aflatoxin by the 1.5% lime solution shows that a higher concentration of lime, and therefore a lower pH, is needed to combat the high stability of aflatoxin. Heat did not seem to have a significant effect on aflatoxin because of its high resistance, but further research into a range of temperatures is needed to make a confident conclusion.

Results of similar studies show results of the same nature. There have been very few studies done to compare aflatoxin levels in maize before and after nixtamalization, but the ones that have been published have come to comparable conclusions. The differences between this study and others lie in the samples tested. There is a large focus on maize in Africa, which is slightly different than the maize varieties native to Mexico that were analyzed in this study. Aflatoxin is also highly variable in analyses, this can create more differences in results depending on how procedures for analysis were performed.

There were many limitations in this study. The primary limitation was time. This research had a very small window to complete data collection which made it difficult because the procedure required a lot of time for each sample. Another limitation was in the aflatoxin itself, aflatoxin are highly variable when performing analysis, so one test of a sample could produce

different results if performed twice. This required each analysis to be diluted or performed again and greatly reduced efficiency. The equipment used in this study was also a limiting factor. The aflatoxin analysis equipment was not functioning properly which required data collection to be halted until the equipment was fixed. Similarly, a piece on the wet-miller equipment used in grinding the nixtamal into masa was broken and required data collection to stop until it was fixed.

This study raises new questions for future work and other solutions to food insecurity. Based on the results of aflatoxin after nixtamalization in different lime concentrations, it would be beneficial to continue testing at different concentrations to determine which is optimal for aflatoxin removal. Nixtamalization, or a process similar, could also be used on different grains contaminated by aflatoxin to discover if this process is effective for other crops. Alternative methods for removal of the pericarp on maize kernels could also be studied because a majority of aflatoxin reside in and around the pericarp. The final question this study raises is how to make nixtamalization a widely used food safety process that is accessible in homes. The results show that nixtamalization is effective at removing aflatoxin from maize, but in order for these results to have an effect on populations, the methods need to be practical for everyday use. Utilizing nixtamalization, malnutrition and food insecurity in populations would start to diminish and the quality of life would drastically increase.

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