Human exposure to and climate influence on Aflatoxin contamination of commercial and cottage industry mesquite (*Prosopis velutina*) pod flour.

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Abstract

Native food enthusiasts in Arizona conduct public millings of wild- and landscape-collected mesquite pods to produce mesquite flour, which is often consumed in the same localities where it is produced without conventional food safety inspection. Aflatoxin contamination of food and feed is a perennial concern in Arizona where aflatoxin contamination, caused mainly by *Aspergillus flavus*, has been previously reported in Fabaceae fruits including *Prosopis* pods. This study identified aflatoxin exposure risk posed by mesquite flour in southeastern Arizona. Aflatoxin was found in both commercial (imported and domestic) and non-commercial mesquite flour batches. Aflatoxin contamination above FDA action levels for human food occurred in 10% of the sampled flour, and only 2 of 37 batches had aflatoxin levels low enough for European export (< 2 ppb). Aflatoxin was also detected in flour imported from Peru and Argentina. Variability in aflatoxin content in Tucson was largely explained by harvest date (63%) with those harvested later in the monsoon season yielding more aflatoxin. Pods harvested in Tucson before monsoonal rains (≤4.5 mm rainfall) produced mesquite flour with aflatoxin below FDA action limits, while those exposed to monsoonal precipitation levels (60-67 mm rainfall) were contaminated with 37 ppb aflatoxin on average. Immunochromatographic lateral flow assay of aflatoxin in mesquite flour proved a viable option for testing in the lab and at public events.

Introduction

A component of regional Indian and South American diets and eaten by indigenous peoples of the Sonoran desert for generations, mesquite (*Prosopis* spp.) has been proposed as a food crop in arid and developing countries because these nitrogen-fixing trees require few inputs, including water. Mesquite pods can be low in antinutrients such as tannins and trypsin inhibitors, and high in antioxidants, protein, and soluble and insoluble dietary fiber. Mesquite can be used to replace wheat in traditional recipes, with tasters preferring recipes containing 40-45% mesquite flour. Arizona producers sell mesquite flour internationally online, in grocery stores, and at farmer's markets with cost per kilogram from 22 to 35 dollars. Mesquite pods are harvested from the wild, landscaping plants (public and private), and commercial orchards.

Aflatoxin contamination of food and feed produced in Arizona is a perennial concern, and susceptible crops are regularly rejected from premium markets due to aflatoxin contamination. Aflatoxins are toxic metabolites produced by fungi in *Aspergillus* section *Flavi*, and aflatoxin B_1 is considered the most carcinogenic naturally occurring compound. The only previous study conducted to address aflatoxin-contamination in mesquite frequently isolated *Aspergillus flavus* and found dangerously high levels of aflatoxin in pods from tree canopies and the ground in native areas.

Study goals were to:

1) assess aflatoxin exposure risk to consumers of mesquite flour in the Tucson area (both from large-scale and cottage industries)

2) determine factors that increase aflatoxin contamination in mesquite products, and

3) evaluate immunochromatographic lateral flow testing for use by small scale producers of mesquite flour.

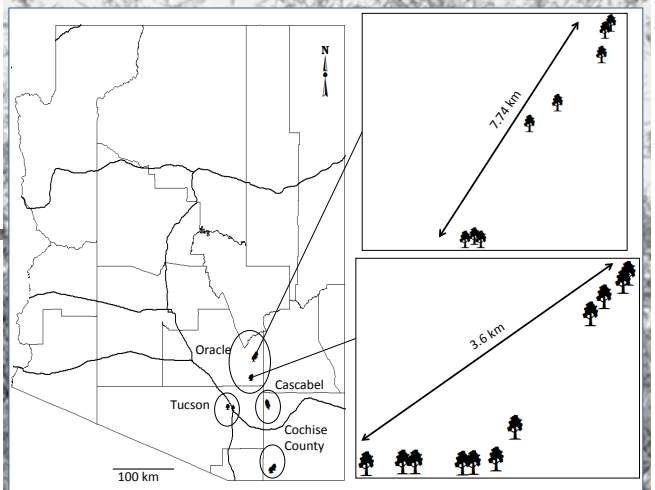

Figure 1. Sampling locations in southeastern Arizona. 4 to 10 samples were collected at each location before and after monsoonal weather patterns brought summer rains. GPS data was collected at each harvest site to represent the number of trees and distance covered for each harvest (inset).

Results

All samples had detectable levels of aflatoxin (Table 1). Only two of the lots of mesquite flour had an aflatoxin concentration low enough for export to Europe (below 2 ppb; one from Tucson and another from Oracle). Two samples from Tucson had aflatoxin content of 24 and 74 ppb, and two samples from Oracle had 48 and 110 ppb aflatoxin, over FDA action limits.

Extraction of 50 g samples of flour allowed useful and reproducible estimates of the flour lot's aflatoxin content. Mesquite processors should be discouraged from using smaller samples which will result in less accurate estimates.

Ten percent of mesquite flour lots evaluated in the current study tested positive for levels of aflatoxin in excess of the FDA action level for human food (Table 1).

Mesquite flour produced from pods harvested in the same vicinity varied widely in aflatoxin contamination, with aflatoxin content of pods harvested 11 days apart ranging from 4 to over 100 ppb (Table 1, Fig. 2). Mesquite flour produced from the Oracle harvest provided the lowest (1.3 ppb) and the highest (110 ppb) aflatoxin levels detected in the current study. This variability continues to show the need for quantification of aflatoxins in mesquite flour.

Mesquite pods present a unique aflatoxin exposure risk for consumers in the US as food produced and consumed locally may fall through the cracks of food hygiene programs. Imported food and food from large producers is routinely scrutinized for aflatoxins, among other food borne pathogens and contaminants (136), while local food from small producers might evade government food hygiene programs because of the proximity of producer to consumer.

For its ease and accuracy (91% recovery), immunochromatographic lateral flow aflatoxin testing would provide assurance on the safety of mesquite based foods in local markets in a number of geographic areas.

In Tucson, harvest date explained 63% of the variability of aflatoxin contamination in samples ($F_{1,8}$=12.03, P=0.0085; Fig. 2), and those pods that were harvested after monsoonal weather patterns had significantly higher levels of aflatoxin ($F_{1,8}$=15.84, P=0.0041; Fig 3).

In the course of performing this study, two community stakeholders with no scientific background were satisfactorily trained in the performance of lateral flow testing on mesquite flour. This assures feasibility of using lateral flow testing at community milling events and by small stakeholders to provide food safety information to consumers, without regulatory intervention.

Materials and Methods

Sampling Plan. Sample locations were chosen among sites where annual or semiannual mesquite pods harvests have regularly occurred and included private and public landscapes, commercial orchards, and wilderness at a variety of elevations (Fig. 3.1). Sites were located at elevations of 285 m to 1615 m above sea level (Table 3.1). Each sample consisted of one 20 liter bucket filled with pods from 20-40 *P. velutina* trees. After harvesting, pods were laid on tarps and dried in the sun until easily broken, then stored in buckets with sealed lids outdoors or left open indoors. Commercial samples were collected from health food and specialty stores, farmer's markets, and online, either located in the sampling area or available to consumers within the sampling area, and were purchased in 227 g and 453 g (0.5 and 1 pound) quantities.

Sample Milling. Pods were pulverized using a gas-powered Number 5 hammer mill (Meadows Mills, North Wilkesboro, NC, USA) fitted with a number 4 screen. Two 200 g samples were collected from each batch of flour produced from each 20 L bucket of pods. Samples were stored at -20°C until analyzed for aflatoxin and moisture content.

Moisture Analysis. Twenty grams of each mesquite flour sample was weighed in pre-weighed metal containers (metal container, 4 oz., Ben Meadows, Janesville, WI, USA) and dried in a gravity convection oven (70°C, 3 d, DX300, Yamamoto Scientific America Inc., San Francisco, CA, USA). Containers were sealed as they were removed from the oven, allowed to equilibrate to room temperature and then weighed (Top Loading Balance, GF-2000 A & D Company Ltd., San Jose, CA, USA). The dry flour weight was obtained by subtracting the container weight, and the percent moisture was obtained by subtracting the flour dry weight from the initial weight of the flour and dividing that difference by the initial flour weight.

Aflatoxin analyses. Neogen Reveal Q+ for aflatoxin (Neogen Corp., Lansing, MI, USA) was selected for use in this study specifically because of room temperature storage. For each sample, 50 g mesquite flour was weighed directly into 500 ml media bottles; 250 ml 65% ethanol was added to each bottle. The mixture was placed on an orbital shaker for 3 minutes. Extracts were allowed to settle, before pouring through fluted #4 filter paper. At least 50 ml of filtrate was collected in a polyethylene beaker, of which 100 µl was mixed with 500 µl test kit diluent, and 100 µl of this diluted filtrate was transferred to the test well. One test strip was dropped into the test well and allowed to develop for 6 minutes, after which the strip was read by the Neogen AccuScan III Reader. Aflatoxin concentration was reported in parts per billion, and the final concentration was adjusted by the percent recovery from the spike and recovery assay. Assay accuracy was corroborated with a spike and recovery assay, which yielded 91% of spiked aflatoxin.

Precipitation measurement. Estimates of precipitation on mature mesquite pods in Tucson were calculated using data recorded by the nearest AZMET weather station (The University of Arizona, Tucson) from the first of June until the harvest date for each sample collected in Tucson.

Table 1. Mesquite flour harvest data and aflatoxin levels

Source	Samples (n)	Elevation (m)	Harvest Days (day number)	% Moisture (w/w)	Aflatoxin (ppb)	Range	# Samples >20 ppb aflatoxin	# Samples >5 ppb aflatoxin*
Tucson	10	730-805	167-202	6	13.5	1.6-81	2	3 A
Oracle	8	714-1317	177-299	5	24.6	1.4-110	2	3 A
Cascabel	4	964	196-246	5	3.9	2.7-5.5	0	1 B
Cochise County	5	1438-1615	213-242	6	3.45	3.2-3.8	0	0 B
Commercial	8	unknown	unknown	4	8.2	5.2-10.4	0	8 A

*Numbers not followed by the same letter are independent (P = 0.0003) using Pearson's χ^2 test.

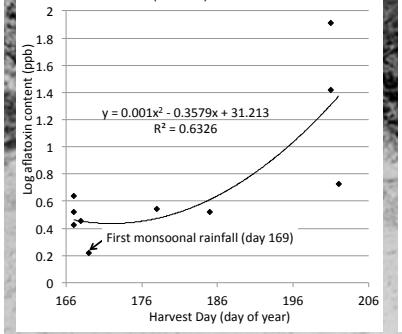

Figure 2 Aflatoxin contamination levels in mesquite flour produced from pods harvested in Tucson is presented to show the statistically significant correlation determined by sum of squares F-test ($F_{2,7}$=6.026, P=0.0301) between harvest day and aflatoxin contamination. A two-degree polynomial trend line was best fit to the data (r^2 = 63.2).

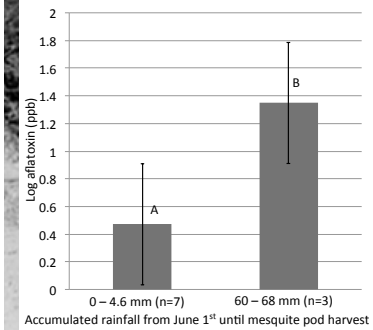

Figure 3 Average log aflatoxin was significantly different ($F_{1,8}$=15.84, P=0.0041) for flour produced from pods exposed to between zero and 4.5 mm rainfall, and those exposed to between 60 and 68 mm rainfall. Letters A or B indicate that log aflatoxin content was different according to Tukey's HSD test (α = 0.05). Rainfall was measured June 1 when the pods were ripe to the harvest date.

Mesquite Pod Harvest and Flour Production Guidelines:

1. Harvest pods as soon as they are ripe, and harvest before monsoonal rainfall at lower elevations (below 1000 m above sea level).

2. Only harvest mesquite pods from the tree. Do not collect pods from the ground.

3. Do not wash pods with water.

4. Dry mesquite pods immediately after harvest and store in sealed food-safe container until milling.

5. Mill pods into flour as soon after harvesting as possible.

6. Aflatoxin is highly variable in mesquite flour and testing is recommended.

Desert Harvesters' Meadows #5 gas-powered, portable hammer mill at a public milling event in downtown Tucson. Community members bring their own mesquite pods and volunteers operate the mill. Pods are passed through a #4 screen and flour is collected in the first barrel and fines are collected above the cyclone.