EVALUATION OF SMALL-SCALE EXTRUSION FOR AFLATOXIN DECONTAMINATION OF MAIZE IN KENYA

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Aflatoxins, secondary metabolites produced by the molds *Aspergilllus flavus* and *A. parasiticus*, are estimated to affect upwards of 25% of the world's global food supply. For Low and Middle-Income Countries like Kenya, a combination of trade, economic, and health challenges related to aflatoxin contamination present a serious threat to food and national security. One option for reducing aflatoxin risks in countries like Kenya is deploying small-scale, reprocessing technologies that degrade aflatoxin in contaminated food products. One potential technology for reprocessing is small-scale extrusion (60 pph) like the TechnoChem Mini-Extruder™.

First, to understand the extent of aflatoxin contamination in Kenyan maize, two field work trials were conducted in Uasin Gishu County, Kenya. Aflatoxin levels from each sample were analyzed and compared to a variety of agro-economic variables (e.g. farm size) using a stepwise multiple linear regression. Upon analysis, only 5% of maize samples collected during field work tested positive for unsafe levels of aflatoxin (>10 ppb). Thus, the resulting regression model is highly biased towards predicting low aflatoxin levels. Such bias makes any inferences to predict high aflatoxin levels in maize largely inconclusive. The inherent heterogeneity of aflatoxin and the history of wide-spread contamination in Kenya further supports the conclusion that more studies are needed to understand the true extent of aflatoxin contamination in Uasin Gishu maize.

Second, to test the effectiveness of small-scale extrusion on aflatoxin degradation in maize, contaminated samples were processed at varying motor frequencies (15, 38, and 50 hz) and moisture contents (35, 40, 45 %wb). Moisture content is significant (p-value < 0.05) in aflatoxin degradation. Total aflatoxin degradation varied between 11 and 83% depending on processing conditions. Maximum degradation occurred at 40 %wb product moisture with a residence time of 265.1 s and an effective shear rate of 56.5 1/s. Thermal degradation is considered negligible due to low temperature increases. Consequently, all degradation is attributed to shear forces inside the extruder. Shear rates were approximated using the Harper model with moisture content and residence time being the most significant factors affecting shear effects on aflatoxin degradation. Although significant aflatoxin degradation occurred in the extruder, further studies are necessary to understand the role of processing parameters on aflatoxin degradation before small-scale extrusion can be confirmed as a viable reprocessing technology.

1. INTRODUCTION

1.1 Project Significance

Aflatoxins, secondary metabolites produced by the molds *Aspergilllus flavus* and *A. parasiticus*, are estimated to affect upwards of 25% of the world's global food supply (WHO, 2018). Specifically, aflatoxins pose a serious threat in foods like maize (corn), tree nuts, and dried fruit, where wet soil and high temperatures can encourage microbiological deterioration especially during harvesting. Poor post-harvest handling also promotes mold growth, particularly when crops are stored in non-hermetic conditions. The outcome of mold contamination is reduced crop yield as well as increased aflatoxin contamination in food and feed products. As a type of highly toxigenic mycotoxin, aflatoxins also pose a serious risk to human health in the form of chronic illnesses and disease.

Low and Middle Income Countries (LMICs) are particularly strained by the burden of aflatoxin contamination in food products. Not only are contaminated crops lost during the production phase, strict global trade tolerances also frequently result in the rejection of shipments from LMICs lacking the ability to comply with such stringent aflatoxin regulations. In 2001, at the Third Conference for Least Developed Countries, Kofi Anan, former Secretary General of the United Nations, estimated that European aflatoxin regulations cost the continent of Africa $670 million each year (Wu & Guclu, 2012). Additionally, aflatoxins have created a public health crisis in many LMICs. The diets of many populations living in LMICs are often comprised of foods at high risk for aflatoxin contamination, such as maize (corn), peanuts, dried fruits, and milk (Ek, Ka, & Kang, 2009; Strosnider et al, 2006). Consequently, these same populations also suffer from aflatoxins most adverse health effects, such as hepatocellular carcinoma (liver cancer) and acute aflatoxicosis (Kaur, Jha, Sabikhi, & Singh, 2014; Mutegi, Cotty, & Bandyopadhyay, 2018b). Cases of aflatoxicosis have also been reported in livestock in these regions (Nyangaga, 2014; Wu & Guclu, 2012).

For LMICs, the combination of trade, economic, and health challenges related to aflatoxin contamination present a serious threat to national food security. Wealthy countries like the United States can provide ample financial support to their agriculture industries and also benefit from a robust agricultural private sector. In contrast, LMICs lack the necessary infrastructure and

financial resources needed to mitigate aflatoxin contamination in major food crops like maize. Such deficits are exacerbated by poor growing conditions, limited education on aflatoxins, and a shortage of affordable storage, handling, and processing technologies for farmers (Yard et al., 2013). Additionally, as much as 80% of the population in many LMICs are engaged in subsistence farming, where commercial food safety regulations do not apply and mitigation technologies are largely inaccessible (Daniel et al., 2011).

The country of Kenya grows and produces a variety of products that are afflicted by aflatoxins, including maize, peanuts (groundnuts), wheat, barley, and milk products. Since the early 2000's, many studies have consistently shown aflatoxins in a variety of food and feed products from various regions in Kenya (Mutegi, Cotty, & Bandyopadhyay, 2018a). The results of these studies are concerning given the number that found aflatoxin levels to be higher than the regulatory threshold of 10 ppb for total aflatoxins and 5 ppb for Aflatoxin B1 (Gong et al., 2015). There is a positive correlation between the frequency of aflatoxin-producing fungi and the consumption of aflatoxins via maize and peanuts (Mutegi et al., 2018a). Of greatest concern is the number of studies finding high aflatoxin levels in staple food products such as maize, since Kenya's per capita maize consumption is one of the highest in the world at an estimated 103 kg/person/year (CIMMYT, 2015). In fact, during field work for this research (Fall 2019) there were five major maize flour brands recalled for unsafe aflatoxin levels (Cheruiyot, 2019). In Spring 2020, 17 maize flour brands were removed from Kenyan supermarkets as the result of aflatoxin contamination (Daily Nation, 2020).

With one of the world's fastest growing and youngest populations, ensuring food safety and health in Kenya is of critical national security, economic, and public health concern. However, the availability of affordable, accessible, and culturally-appropriate aflatoxin mitigation and reprocessing technologies is limited. While there are many available innovations suitable for decontaminating aflatoxin-infected foods, a variety of agroeconomic challenges prevent Kenyan producers and processors from adopting such technologies. For processors, such as large flour mills and traders, the cost of aflatoxin testing (minimum 5 USD/test) is a barrier to ensuring food safety for at-risk products. Many private companies ultimately choose not test their products appropriately before releasing them into the public market. Subsistence farmers and smaller communities are particularly susceptible to aflatoxin exposure, since commercial food safety regulations do not apply and mitigation technologies are largely inaccessible at the local level

(Daniel et al., 2011). Employing any aflatoxin mitigation is also largely infeasible given the low income elasticity of subsistence farmers who already face challenges affording quality crop storage and agricultural inputs. The compounding detriments of climate change, which continues to create more unideal and unpredictable agricultural conditions, further exacerbates farmers' inability to control aflatoxin levels (Strosnider et al., 2006).

There are a variety of industrial reprocessing methods for contaminated maize products, which include boiling, roasting, sorting, and some chemical treatments (Jalili, 2015; Kabak, 2009a; Samarajeewa, Sen, Cohen, & Wei, 1990). However, a majority of these reprocessing methods remain largely inaccessible in LMICs, where such methods are often expensive and have limited applicability. One potentially affordable and feasible technology for reprocessing in Kenya is small-scale extrusion. As a conventionally high temperature, short time (HTST) processing method, extrusion is known for its ability to maintain food quality while improving food safety and bioavailability. Research thus far has proven that industrial sized extruders (1000 kg/hr) can significantly reduce aflatoxin levels (Bullerman & Bianchini, 2007; Elias-Orozc, Castellanos-Nava, Gaytan-Martinez, Figueroa-Cardenas, & Loarca-Pina, 2010; Kabak, 2009a; Firibu Kwesi Saalia & Phillips, 2011). However, little research has been done to support the use of small-scale extrusion processing and determine its feasibility – both technically and culturally – as a viable aflatoxin reprocessing method in developing countries.

Currently, a small-scale extruder known as the mini-Extruder™ (TechnoChem International) has been deployed by United States Agency for International Development (USAID) Feed the Future initiative at multiple Food Process Innovation Labs (FPILs) across the globe. At the Institut de Technologie Alimentair (ITA) in Senegel, the mini-Extruder™ is used to make instant couscous. In Kenya, the mini-Extruder™ is at the University of Eldoret's Food Processing Training and Incubation Centre (FPTIC) where it is used to create an instant, fortified porridge flour using locally sourced vegetables and grains.

1.2 Project Objectives

The overall goal of this study was to identify if the mini-Extruder™ is an appropriate technology for decontaminating aflatoxin-infected maize in Kenya. The specific objectives were to:

1. Conduct a case study in Uasin Gishu County, Kenya to determine the extent of aflatoxin contamination in maize samples and identify the applicability of extrusion reprocessing technology in the maize supply chain.
2. Determine if small-scale extrusion is a viable method for reprocessing aflatoxin-contaminated maize. And, if so, what conditions allows for the highest total percent degradation.
3. Understand the effects of extrusion processing parameters on aflatoxin degradation in maize and make design recommendations for future small-scale extruders.

2. A REVIEW OF AFLATOXIN CONTAMINATION IN KENYA AND JUSTIFICATION FOR EXTRUSION AS A REPROCESSING METHOD

2.1 Introduction

Aflatoxins, secondary metabolites produced by the molds *Aspergilllus flavus* and *A. parasiticus*, are estimated to affect upwards of 25% of the world's global food supply (WHO, 2018). Specifically, aflatoxins pose a serious threat in foods like maize (corn), tree nuts, and dried fruit, where wet soil and high temperatures can encourage microbiological deterioration especially during harvesting. Poor post-harvest handling also promotes mold growth, particularly when crops are stored in non-hermetic, high-moisture, and high-temperature conditions. The outcome of mold contamination is reduced crop yield as well as increased aflatoxin contamination in food and feed products. As a type of highly toxigenic mycotoxin, aflatoxins also pose a serious risk to human health in the form of chronic illnesses and disease (Bhunia, 2008).

As a potential detriment to both the agriculture industry and public health, aflatoxins are highly regulated at an international and national level. At the international level, the Codex Alimentarius Commission (Codex) prescribes minimum levels (MLs) for total aflatoxins in foods like peanuts and some tree nuts (e.g. Brazil nuts) as well as an ML for M1 aflatoxin in milk. These MLs are generally respected by global entities, such as the Food and Agricultural Organization (FAO) and the World Health Organization. While Codex does not outline MLs for all crops contaminated by aflatoxin (e.g. maize and rice), many governing bodies, such as the European Union and individual nations, have set their own regulations for such food products in an attempt to mitigate health risk for consumers. For example, the European Union (EU) has a strict ML of 4 ppb for AFB1 in food products. Such low MLs can also present serious trade barriers and incur additional costs for producers, processors, and traders (Gong et al., 2015).

For Low and Middle Income Countries (LMICs) the combination of trade, economic, and health challenges related to aflatoxin contamination present a serious threat to national food security. Wealthy countries like the United States can provide ample financial support to their agriculture industries and also benefit from a robust agricultural private sector. In contrast, LMICs like Kenya lack the necessary infrastructure and financial resources to mitigate aflatoxin contamination in major food crops like maize. Such deficits are exacerbated by poor growing

conditions, limited education on aflatoxins, and a shortage of affordable post-harvest technologies for farmers (Yard et al., 2013). Additionally, like many other LMICs, much of Kenya's population works full or part-time in subsistence farming, where commercial food safety regulations do not apply and mitigation technologies are largely inaccessible (Daniel et al., 2011).

In the past decade, many studies have highlighted the negative affect of aflatoxin on public health and food security in LMICs (Kumar, Mahato, Kamle, Mohanta, & Kang, 2017; Kussaga, Jacxsens, & Luning, 2014; Mahato et al., 2019; Strosnider et al., 2006). As a result, many LMIC governments and private industries have invested in aflatoxin mitigation and prevention methods. In Kenya, there has been a focus on preventing aflatoxin contamination at the production level through extension education with farmers as well as improved monitoring and surveillance by the Kenya Bureau of Standards (KEBS). While these efforts help to control aflatoxin at the production level and are critical for preventing aflatoxin contamination, implementing novel reprocessing methods for contaminated foods is also necessary to build a robust food supply chain.

There are a variety of reprocessing methods for contaminated maize products, which include boiling, roasting, sorting, and some chemical treatments (Jalili, 2015; Kabak, 2009a; Samarajeewa et al., 1990). However, a majority of these reprocessing methods remain largely inaccessible in LMICs, where such methods are often expensive and have limited applicability. Some solutions may work for large scale producers, but fail to reach more vulnerable populations like small-holder farmer communities. In Kenya, many small-holder farmers resort to feeding moldy maize to livestock. While this may prevent individuals from directly consuming the contaminated food, the risk is minimally reduced as livestock convert aflatoxin into its M1 form which is still toxic when ingested. Thus, an affordable and accessible reprocessing method is necessary to further reduce consumers' risk of exposure to aflatoxin in susceptible food products.

Small-scale extrusion presents a viable reprocessing method for aflatoxin-contaminated crops like maize and peanuts. Small-scale extrusion has been proven to have many applications for small-holder food processing entrepreneurs in developing countries (Penner, 2011; Ponrajan, 2016). Currently, the mini-Extruder™ has been deployed by USAID Feed the Future at multiple Food Process Innovation Labs across globe. At the Institut de Technologie Alimentair (ITA) in Senegel, the mini-Extruder™ is used to make instant couscous. In in Kenya, the mini-Extruder™ is at the University of Eldoret's Food Processing Training and Incubation Centre (FPTIC) to create an instant, fortified porridge flour.

As a conventionally high temperature, short time processing (HTST) method, extrusion is known for its ability to maintain food quality while improving food safety and bioavailability. This aspect of extrusion extends to mycotoxins, which are usually difficult to destroy by traditional food processing methods. Research thus far has proven that industrial sized extruders provide enough shear and temperature to significantly reduce aflatoxin levels (Bullerman & Bianchini, 2007; Elias-Orozc et al., 2010; Kabak, 2009a; Firibu Kwesi Saalia & Phillips, 2011). However, little research has been done to support small-scale extruder processing and its effect on aflatoxin contamination in the context of developing country food systems.

This review serves to present the current state of aflatoxin contamination in Kenya and a justification for small-scale extrusion as means of reprocessing aflatoxin-contaminated foods. To begin, a brief chemical and pathogenic review of aflatoxin is provided. This is followed by a review of the current state of aflatoxin occurrence in Kenya, where particular attention is given to the Kenyan socio-economic and cultural framework in which aflatoxin reprocessing technology must be successful. This is followed by a detailed assessment of extrusion technology and its effect on aflatoxin degradation.

2.2 Aflatoxin Description

Mycotoxins are secondary fungal metabolites that contaminate many agricultural commodities like maize (corn) and tree nuts (e.g. peanuts) (Strosnider et al., 2006). Aflatoxin is a naturally occurring mycotoxin produced by the molds *Aspergillus flavus* and *Aspergilllus parasiticus*. Ideal conditions for mycotoxin production occur at a water activity level between 0.85 and 0.99 and temperatures ranging from 10 – 30 °C. Poor agricultural conditions, like wet soil and high temperatures, encourage microbial deterioration and the production of aflatoxins. Later, during the transportation and storage stages of production, microbiological deterioration and mold growth continue when crops are stored under non-hermetic, high-moisture, and high-temperature conditions (Bhunia, 2008).

Aflatoxin is a secondary metabolite with a low molecular weight. The ring structure of aflatoxin, as seen in Figure 2.1, makes it highly heat stable and difficult to destroy using traditional processing methods. There are at least 13 different types of reported aflatoxins, but aflatoxin B1 (AFB1) is considered the most toxic and is classified as a Group I carcinogen by the International

Agency for Research on Cancer (IARC). When discussing total aflatoxin levels, this is usually in reference to the sum of four sub-types of aflatoxin: AFB1, AFB2, AFG1, and AFG2. The B and G prefixes represent whether a certain strain glows blue or green respectively under ultraviolet light. Aflatoxin M1 (AFM1), the converted form of AFB1, is also toxic and found in milk products (Bhunia, 2008).

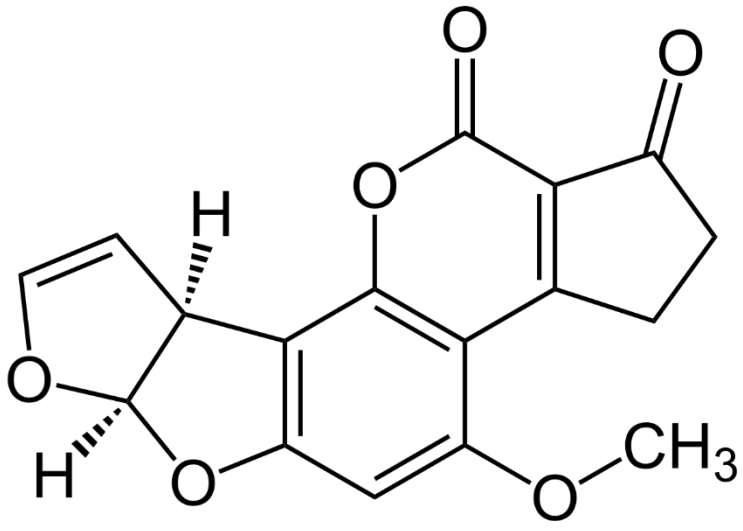

Figure 2.1: The chemical structure of Aflatoxin B1.

The consumption of aflatoxin is related to a number of health concerns in humans and animals. The lethal dose for humans is ~10-20 mg of aflatoxin, but it is important to note that aflatoxin also accumulates in the body with time (Bhunia, 2008). Aflatoxins are potent carcinogens and long-term consumption is known to be the cause of various chronic diseases including organ failure, cancer, and jaundice. The target organ of aflatoxin pathogenesis is the liver, where the mitochondrial cytochrome P450 enzyme converts aflatoxin into a reactive 8,9-epoxide form that can bind to DNA and lead to GC to TC amino acid transversions. These DNA transversions often lead to carcinogenesis, causing liver damage as well as colon and liver cancer. Long-term exposure to aflatoxin has also been linked to birth defects, carcinoma, skin irritation, and neurotoxicity (Bhunia, 2008).

Consuming a large amounts of aflatoxin in a short period of time can also result in acute aflatoxicosis in animals and humans. Outbreaks of acute aflatoxicosis have been recorded worldwide, often in regions where socio-economic barriers prevent proper aflatoxin mitigation and control. Strosnider et al linked higher prevalence of hepatocellular cancer in Africa to aflatoxicosis

outbreaks. In 2013, a major outbreak affected populations in Romania, Serbia, and Croatia as well as western European countries receiving crops from those areas (Kumar et al., 2017). Researchers have found aflatoxins in the umbilical blood of infants in Nepal and Bangladesh as a result of ongoing exposure (Groopman et al., 2015). Outbreaks of aflatoxicosis in Kenya have occurred as recently as 2014 with the most severe outbreak occurring in Eastern Kenya in 2004 (Mutegi et al., 2018b).

2.3 Occurrence of Aflatoxin in Kenya

Purdue University has extensive partnerships with different universities, research groups, non-governmental organizations, and governmental bodies conducting international development work in Kenya. It follows that Purdue and the University of Eldoret (Eldoret, KE) have partnered to address the issue of aflatoxin contamination in the Kenyan food and feed supply chain. In Section 2.3, the past and current state of aflatoxins in Kenya is discussed to provide a framework for the field work and technical research conducted as part of this thesis.

2.3.1 Aflatoxins in Food and Feed

The country of Kenya grows and produces a variety of products that are afflicted by aflatoxins, including maize, peanuts (groundnuts), wheat, barley, and milk. Since the early 2000's, many studies have consistently shown aflatoxins in a variety of food and feed products from various regions in Kenya (Mutegi et al., 2018a). The results of these studies are concerning given the number that found aflatoxin levels to be higher than the regulatory threshold of 10 ppb for total aflatoxins and 5 ppb for aflatoxin B1 (Gong et al., 2015). Of greatest concern is the number of studies finding high aflatoxin levels in staple food products such as maize and peanuts. Kenya's per capita maize consumption is estimated at 103 kg/year (CIMMYT, 2015). Street peanuts are frequently consumed as a snack food by many Kenyans, particularly children and frequent travelers. There is a positive correlation between the frequency of aflatoxin-producing fungi and the consumption of aflatoxins via maize and peanuts (Mutegi et al., 2018a)

High aflatoxin M1 levels have also been recorded in Kenya. Kenyans have the largest consumption of raw milk (110 liters/person/year) in sub-Saharan Africa (Rademaker, Omedo Bebe, van der Lee, Kilelu, & Tonui, 2016). While M1 aflatoxin is not as toxic as B1, its ability to persist

in a variety of milk products increases exposure. It is also observed that many animals are fed contaminated grain due to lack of knowledge regarding grain disposal methods and the pathogenesis of aflatoxin. Farmers are unaware that the contamination can spread from the grain to the milk product via livestock feed. Rural and low-income communities are at particularly high risk since most milk is sourced informally, which can perpetuate and prolong aflatoxin exposure (Mutegi et al., 2018a). The popularity of such food products susceptible to aflatoxin contamination contributes to high aflatoxin exposure rates. A cross-sectional study conducted in 2007 on aflatoxin exposure in Kenya revealed that 78% of serum samples had detectable levels of aflatoxins (Yard et al., 2013)

The national government provides data and monitoring of aflatoxin outbreaks in Kenya. However, the robustness and effectiveness of government monitoring and surveillance is limited by a variety of socio-economic and political factors (e.g. lack of consistent regulation enforcement) (Gong et al., 2015). Additionally, the cost of aflatoxin testing (minimum 5 USD/ test) limits the effectiveness of many private and public aflatoxin interventions. However, in recent years, outbreaks of acute aflatoxicosis and studies linking increased cancer rates with aflatoxin exposure in Kenya have reinvigorated the public and private sectors' commitment to aflatoxin control (Mutegi et al., 2018a). In fact, during the course of field work for this research (Fall 2019) there were five major maize flour brands recalled for unsafe aflatoxin levels (Cheruiyot, 2019). In Spring 2020, 17 maize flour brands were removed from Kenyan supermarkets as the result of aflatoxin contamination (Daily Nation, 2020). Not only did this reiterate the importance of aflatoxin research, it also demonstrates a growing concern within the Kenyan government to reduce the incident of aflatoxin exposure.

A key part of aflatoxin prevention and mitigation is understanding the complex food supply chain that exists in Kenya. Figure 2.2 from the International Agency for Research on Cancer (IARC) represents a schematic of the maize supply chain in Kenya. The combination of informal and formal trading, production, and processing makes identifying the source of aflatoxin difficult (IARC, 2012). Large companies, given their capital resources, are at an advantage for controlling and surveilling aflatoxin in their products. Some large grain companies (e.g. Unga Limited) already conduct their own aflatoxin testing with every shipment. However, such companies may source from a range of farms and regions as well as other countries like Uganda. Thus, if a batch tests positive for high aflatoxin levels, it is nearly impossible to determine the true source of

contamination. In addition to the sourcing challenges, it is also possible that contamination occurs on site due to poor drying and storage methods. A majority of grain companies and farmers continue to sun-dry grains using on tarps as seen in Figure 2.3.

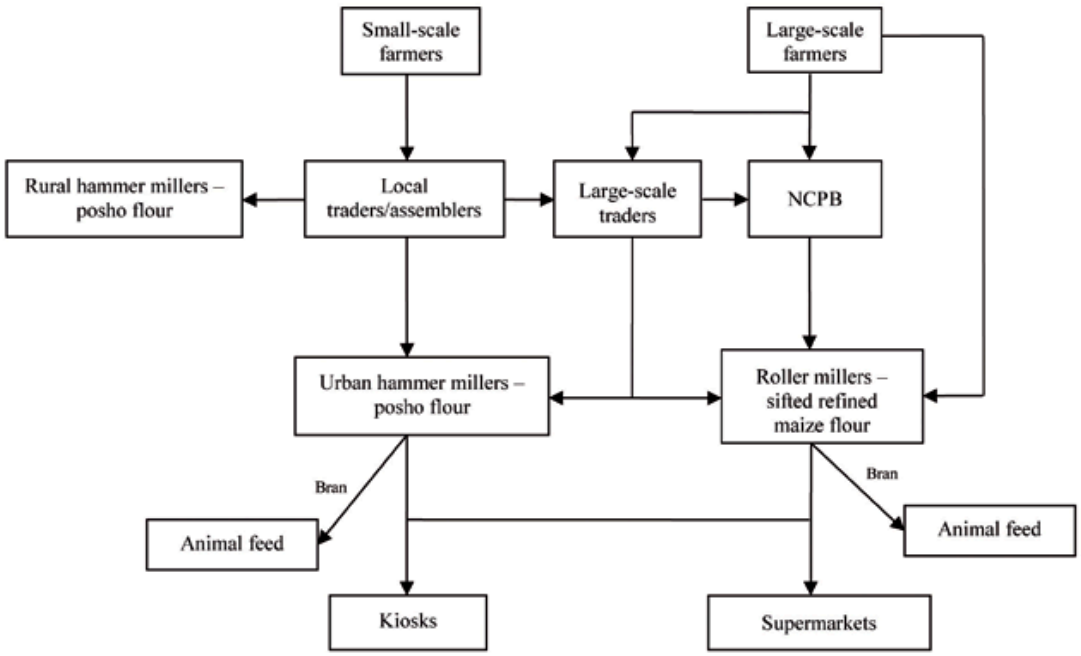

Figure 2.2: The maize supply chain in Kenya. NCPB, National Cereals and Product Board of Kenya (IARC, 2012).

A significant portion of the maize trade happens informally at the local level. An estimated 75% of Kenyans derive their livelihoods from the agriculture sector in some way, many through growing their own food at home on small family plots (USAID, 2019). Such small-holder farmers, farming between one and 30 acres of land, bring their maize to local millers known as Poshomoris for processing. Farmers and their family most often sell their remaining harvest via local traders to neighboring communities. Local traders often combine the harvest of many farmers when selling maize, replicating the tracing issue experienced by larger companies. Consequently, the contamination of a single farmer's crop can affect a large number of people within the surrounding community.

While local and county governments do utilize extension programs to educate and assist local farmers, they often lack the necessary funds to provide long-term and consistent education. Additionally, the complexity of the food value chain in Kenya makes it difficult to implement aflatoxin mitigation strategies. In the event of an outbreak, communication among different stake

holders (e.g. farmers, traders, and millers) would be critical. However, implementing interventions may be only marginally effective given certain socio-economic constraints that limit the coordination of resources (Mutegi et al., 2018a). Thus, the complexity of the local supply chain places many local communities in Kenya at risk for high aflatoxin exposure.

Figure 2.3: Sun drying of wheat in Uasin Gishu County Kenya (Hegwood, 2019).

2.3.2 Aflatoxins and Public Health

Aflatoxin is related to a number of health concerns in humans and animals. It was first suggested in 1962 that aflatoxin ingestion could lead to liver cancer. Since the 1960s, extensive public health and medical studies have been completed to research the effect of aflatoxin on public health and well-being (Mutegi et al., 2018a). The target organ of aflatoxin pathogenesis is the liver, where the mitochondrial cytochrome P450 enzyme converts aflatoxin into a reactive 8,9-epoxide form that can bind to DNA and lead to GC to TC amino acid transversions. These DNA transversions often lead to carcinogenesis, causing liver damage as well as colon and liver cancer. Aflatoxin B1, considered the most toxic of aflatoxin types and is classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC).

The history of aflatoxin-related illness is well recorded in Kenya. In 1967, it was established that the Kamba tribe had twice the frequency of liver cancer as the Kikuyu ethnic community. The same Kamba community, located in Eastern Kenya, has been historically prone to endemic outbreaks of acute aflatoxicosis. It was not until 1981, that the first recorded acute human aflatoxicosis outbreak in Kenya occurred with 12 fatalities. A second major outbreak occurred between 2004 and 2005, again in Eastern Kenya during the cropping season (Mutegi et al., 2018a).

A Kenyan's exposure to aflatoxin begins as early as infancy with newborns drinking breast milk and or the milk from dairy cows. In section 2.2, aflatoxin is described as having the ability to mutate from its most toxic form (AFB1) to its M1 form via digestion of ruminants. The M1 form of aflatoxin can also be found in infants through the consumption of breast milk. A high proportion of Kenyan mothers tested positive for aflatoxin level in breast milk. This, in combination with the prevalence of M1 aflatoxin in dairy milk, shows that aflatoxin can begin when individuals are born and continue with chronic exposure through the rest of their life. Long term exposure and the accumulation of aflatoxin in the body leads to a variety of illnesses, including cancer, birth defects, and acute aflatoxicosis. Aflatoxin contamination has also been linked with malnutrition, which aligns with trends showing aflatoxin levels as higher in low-income regions – the same regions that lack access to safe and affordable food options (Mutegi et al., 2018a).

2.4 Reprocessing Methods for Aflatoxin Contaminated Foods

Aflatoxin decontamination is a continuous challenge for the food industry (Bullerman & Bianchini, 2007; Kabak, 2009b; Milani & Maleki, 2014; Samarajeewa et al., 1990). Aflatoxins can be controlled and prevented first by good agricultural practices, which includes farmers working closely with extension officers to harvest, store, and transport their grain in more effective ways. Additionally, good manufacturing practices (GMP) during pre and post-harvest handling such as soil testing, crop rotation, irrigation, antifungal treatments, appropriate harvesting conditions, proper drying, and storage are also beneficial. However, prevention is not always possible under certain agronomic and storage practices (Samarajeewa et al., 1990). Thus, viable decontamination and reprocessing methods play a critical role in ensuring stable and resilient food supply chains afflicted by aflatoxins.

Both physical and chemical methods can be used to reprocess aflatoxin-contaminated foods (Samarajeewa et al., 1990). At the most basic level, a variety of sorting, trimming, and cleaning methods can be used to remove contaminated food material. In many major trading ports around the world, shipments that test for high levels of aflatoxin often undergo some type of sorting process. However, it is important to note that these physical methods do not destroy aflatoxins. Rather, cleaning and sorting only help remove of extensive mold growth and rid of broken kernels, which are often more susceptible to contamination (Bullerman & Bianchini, 2007). To effectively decontaminate a food product, certain physico-chemical and biochemical changes must take place to significantly decrease aflatoxin toxicity.

Most physical and chemical processes are aimed at destabilizing aflatoxin B1 (AFB1), the most toxic sub-type of aflatoxin. There are two characteristic sites for AFB1. First, a double bond at position 8,9 of the furo-furan ring seen in Figure 1. At this site, aflatoxin- DNA and -protein interactions occur and changes can alter the biochemical functions of the toxin. Second, the reactive site of the lactone ring can be easily hydrolyzed, making it an ideal target for aflatoxin degradation methods (Samarajeewa et al., 1990). It follows that effective decontamination methods are aimed at removing the double bond on the terminal furan ring or opening the lactone ring. Such changes can be achieved by physical changes induced by a supply of energy or chemical changes that block or remove active sites (Samarajeewa et al., 1990).

2.4.1 Physical Methods

There are a variety of physical methods which apply energy to achieve varying levels of aflatoxin decontamination in products. Energy can be applied in the form of heat, gamma radiation, ultraviolet (UV) light, visible light, or shear forces. Heating and various forms of thermal processing are the most extensively studied method of decontamination given their feasibility and widespread use across the food industry. Unfortunately, aflatoxins are unique in their ability to withstand even the high temperatures applied by traditional heating methods such as roasting. The melting point of solid AFB1 is 260 °C (Samarajeewa et al., 1990). Some studies have even recorded that temperatures as high as 300 °C are necessary to effectively degrade aflatoxin (Rustom, 1997).

Samarajeewa et al reports that partial decontamination can be achieved with processing methods of 100 °C or higher. Normal food processing conditions reported resulted in 60% degradation on average (Samarajeewa et al., 1990). For maize products in particular, a 40 – 80% reduction in aflatoxin levels was observed when continuously roasting corn between 145 and 180 °C (Conway, Anderson, & Bagley, 1978). 13% degradation of aflatoxin was achieved when baking corn muffins (Gomma, 1987). Boiling and frying achieved a 28% and 33 - 53% degradation respectively for corn grits (Stoloff & Truckscss, 1981). Microwave treatment at high energy levels can result in as much as 95% destruction of aflatoxin (Chinaphuti, 1999; Herzallah, Alshawabkeh, & Fataftah, 2008). The need for elevated temperatures over long periods of time and high pressures to effectively degrade aflatoxin in contaminated food products makes traditional heat treatment largely impractical. Additionally, such high temperatures often result in the destruction of vital nutrients and lead to poor product quality from burning.

Shear forces also contribute to a various number of chemical reactions and molecular modifications to compounds inside food products, including compounds like mycotoxins (Bullerman & Bianchini, 2007). From literature, it is well known that extrusion processing has the ability to significantly lower and reduce the concentration of aflatoxin in food products (Bullerman & Bianchini, 2007; Elias-Orozc et al., 2010; Kabak, 2009a; Firibu Kwesi Saalia & Phillips, 2011). However, the reduction in aflatoxins in a given food product is dependent on a number of variables including temperature, screw speed, moisture content, and residence time in the extruder (Bullerman & Bianchini, 2007).

2.4.2 Chemical Methods

In industry, chemical methods of degradation of aflatoxins are often perceived as the more practical approach. Many studies have been conducted on chemicals and their ability to detoxify AFB1, including various chlorine compounds and oxidizing agents (Samarajeewa et al., 1990). As previously mentioned, chemical methods are used with the goal of either oxidizing the double bond of the terminal furan ring or hydrolyzing the lactone ring of AFB1 to reduce the toxicity of aflatoxin. While many chemical treatments have proven effective for degrading and destabilizing aflatoxin, it is important to note that mitigating risk for the consumer when such chemical compounds are used is of critical concern for maintaining food safety.

Aqueous chlorine is used throughout the food industry to sanitize equipment and wash raw food products, such as meat, fruits, and nuts Sodium hypochlorite was first used to remove aflatoxins from contaminated surfaces and hardware, but was later found to also be effective in degrading aflatoxins in food products (Karlovsky et al., 2016; Samarajeewa et al., 1990). Chlorination with sodium hypochlorite at varying concentrations (0.2 – 11%) was found to almost completely degrade AFB1 in pure form and contaminated foods except in the case of peanuts, where only a 50% deactivation was achieved (Samarajeewa et al., 1990). While chlorine has proven an effective method of degrading aflatoxin, the effect of residual chlorine residue in treated foods could lead to additional concerns with toxicity, particularly when concentration of chlorine remain in fat and protein.

Hydrogen peroxide (H2O2) also has the ability to degrade aflatoxins, including AFB1. Similar to chlorine, H2O2 at varying concentration (0.5% - 6%) has been proven to almost totally degrade AFB1 in contaminated foods, including peanut products (Samarajeewa et al., 1990). However, at some concentrations, H2O2 still allows for mold growth, which is a key part of aflatoxin production in food products. As such, foods treated with H2O2 may put consumers at an increased risk of aflatoxin consumption at later stages in food processing (Samarajeewa et al., 1990). Ozone, a powerful oxidizing agent that reacts with the furan ring of aflatoxin structure, can effectively degrade AFB1 and AFG1 within a few minutes at room temperature. However, AFB2 and AFG2 have exhibited higher resistance to ozone treatment, suggesting that ozone may not be an effective treatment for total aflatoxin reduction. Sodium bisulfate, a common food additive, is also shown to inactivate aflatoxins products (Karlovsky et al., 2016; Samarajeewa et al., 1990).

Ammonia, in various forms, is the most thoroughly studied method of chemical degradation of aflatoxin. A combination of up to 5% ammonia, 10 – 20% moisture, and temperature-time related combinations has been repeatedly proven effective for aflatoxin degradation (Samarajeewa et al., 1990). At temperatures from 80 – 120 °C, food products must be exposed to for 15 – 30 minutes. For naturally contaminated yellow corn, treatment of 2% gaseous NH3, resulted in 52.7 to 67.7% and 79.4 to 93.1% reduction of AFB1 at 12% and 16% moisture respectively (Weng, Martinez, & Park, 1994). Corn evaluated under similar conditions except with 1.5% ammonia saw degradation of aflatoxins greater than 99% (Samarajeewa et al., 1990).

Ammonia treatments in animal feed products are less effective, but degradation between 77 and 99% can still be achieved under laboratory conditions. An average of 85% reduction in

feed products was achieved in large scale experiments (Samarajeewa et al., 1990). The presence of ammonia and duration of treatment was found to have some beneficial nutritional effects in ammoniated feed, such as an increase in total nitrogens leading to improved amino acid digestibility. However, protein quality as well the availability of nutrients like lysine decreases. Samarajeewa et al notes that ammoniation treatment seem less acceptable when nutritional losses are taken into account.

2.5 Extrusion as a Reprocessing Method for Aflatoxin-Contaminated Foods

As a conventionally high-temperature, short-time processing (HTST) method, extrusion is known for its ability to maintain food quality while improving food safety and bioavailability. Mycotoxins are highly heat stable and difficult to destroy by traditional food processing conditions. Research thus far has proven that industrial sized extruders provide enough shear and temperature to significantly reduce aflatoxin levels (Bullerman & Bianchini, 2007; Elias-Orozc et al., 2010; Kabak, 2009a; Firibu Kwesi Saalia & Phillips, 2011). Small-scale extrusion has been proven to have many applications for small-holder food processing entrepreneurs in developing countries (Penner, 2011; Ponrajan, 2016). Currently, the mini-Extruder™ has been deployed by the USAID Feed the Future at multiple Food Process Innovation Labs across At the Institut de Technologie Alimentair (ITA) in Senegal, the Mini-Extruder™ is used to make instant couscous. In in Kenya, the mini-Extruder™ is at the University of Eldoret's Food Processing Training and Incubation Centre (FPTIC) to create an instant, fortified porridge flour. Small-scale extrusion presents a potentially viable reprocessing method for aflatoxin-contaminated crops like maize.

2.5.1 Overview of Extrusion Processing

Established extrusion processing began in the mid-20th century and now has applications across multiple industries, including polymer, pharmaceutical, and food processing businesses. Extrusion is a conventionally high-temperature, short-time processing operation where a molten or dough-like material is shaped and formed by forcing it through a restriction, or die (Bouvier & Campanella, 2014). Cold extrusion is also used in making pasta. During this process, multiple reactions, such as starch gelatinization, protein denaturation, and network formation can occur.

Each of these reactions helps to shape the characteristics of the final product, resulting in notable changes to both nutritional and functional qualities (Nkama & Bulus Filli, 2006).

Today, extrusion processing technology utilizes two designs: single screw and twin-screw extruders. Both types of equipment have various uses and the optimization of their design is closely linked to their extensive use across various industries (Bouvier & Campanella, 2014). While both types of processing equipment are well-developed, twin-screw extruders are favored throughout the cereal-processing industry and are responsible for the production of many well-known food products such as puffed pastries, expanded snacks, pasta, flat bread, and porridges. Twin-screw extruders are even used to convert cereal grains into livestock and aquaculture feed (Bouvier & Campanella, 2014).

Extrusion is one of the most versatile processing methods available and has the ability to produce nutritionally balanced and enriched foods (Balasubramanian, Kaur, & Singh, 2014). It has high production capacity, versatility, and low cost per product (Almeida-dominguez *et al.*, 1993). Additionally, extrudates can be easily fortified with additives to produce high-nutrient supplementary products (Gowda, Rai, & Reddy, 2008). Currently, extrusion has been used to create various puffed snacks, pastas, vermicelli, *sev*, *fura*, and porridges (Singh & Saini, 2012). These products are ready-to-eat and demonstrate increased nutrient availability, quality functional properties, and improved consumer acceptability in comparison to traditionally processed products (Singh & Saini, 2012). Thus, extrusion provides an opportunity to create new, nutritious, and affordable food products for a wide variety of uses and is an ideal processing method for the development of acceptable and nutritious instant composite flours made from locally available foods.

2.5.2 Role of Extrusion in Decontaminating Aflatoxin-Contaminated Foods

As a high temperature, short time processing method, extrusion deactivates anti-nutritional factors to improve shelf-life and extrudate nutritional quality (Kaur et al., 2014). Extrusion processing brings about numerous reactions, which influence characteristics such physico-chemical properties as solubility, water binding capacity, swelling and apparent viscosity, gel formation, and strength (Bullerman & Bianchini, 2007; Filli, Nkama, Jideani, & Ibok, 2013). Extrusion also plays an important role in food safety. Sumathi *et al* found that extrusion also

renders a product microbially safe for consumption by reducing the anti-nutritional factors like trypsin inhibitors, lipases, tannins, and phytates, all of which inhibit protein digestibility (Jiddere, 2016).

During extrusion cooking, products can reach very high temperatures. High temperatures are achieved by pushing the food product – often a dough-like mixture – through a small channel or barrel via a rotating screw. As a result, high temperatures (>150°C) and shear forces can be generated by the extrusion process (Bullerman & Bianchini, 2007). High temperature and shear forces also contribute to a various number of chemical reactions and molecular modifications to compounds inside food products, including compounds like mycotoxins (Bullerman & Bianchini, 2007). From literature, it is well known that extrusion processing has the ability to significantly lower and reduce the concentration of aflatoxin in food products (Bullerman & Bianchini, 2007; Elias-Orozc et al., 2010; Kabak, 2009a; Firibu Kwesi Saalia & Phillips, 2011). However, the reduction in aflatoxins in a given food product is dependent on a number of variables including temperature, screw speed, moisture content, and residence time in the extruder (Bullerman & Bianchini, 2007).

To destroy or inactivate aflatoxins, extrusion cooking conditions need to be severe (Firibu Kwesi Saalia & Phillips, 2011). Such severe conditions are achieve through high shear, high temperature, and a high pH. As in the case of any food processing, the actual extrusion conditions required to ensure appropriate nutritional and sensory quality also depend on the food ingredients (Akande, Nakimbugwe, & Mukisa, 2017). From extrusion, the greatest reduction in mycotoxin concentrations seem to occur at temperatures greater than 160 °C and with longer residence times (Bullerman & Bianchini, 2007). However, such severe conditions can have detrimental effects on the nutritional quality of extrudates. Saalia and Phillips found that extrusion conditions reduced protein availability. Extrusion cooking is less effective with naturally contaminated peanut meal than artificially contaminated peanut meal (Firibu Kwesi Saalia & Phillips, 2011). The effect of extrusion processing on aflatoxins can also be influence by the presence or absence of different chemical additives (Bullerman & Bianchini, 2007). With the addition of ammonia, extrusion processing was able to achieve a 95% reduction in comparison to the 50 -80 % achieved without ammonia (Samarajeewa et al., 1990).

The reduction in aflatoxin is part of extrusion processing's greater ability to render products as microbially safe for consumption through the reduction of anti-nutritional factors (e.g. molds).

Such microbial changes also prolong the shelf life of products (Sumathi, Ushakumari, & Malleshi, 2007). Nkama and Bullus Filli found that Nigerian extruded fura can store for up to six months in low-density polyethylene and cellophane packaging materials when stored at 30 ˚C. These products were also found acceptable to the consumer – even after 12 weeks of storage. Sumathi et al also recorded that the shelf life of extrudates was as great as six months in different flexible pouches under ambient storage conditions. However, when products were not stored under optimal conditions shelf life dramatically decreased. Mold became visible in samples stored at 80 and 90% humidity within just two weeks of storage. This reiterates the need for dry, cool storage conditions and a type of non-permeable packaging even after extrusion processing (Sumathi et al., 2007).

3. EVALUATING AFLATOXIN CONTAMINATION IN WESTERN KENYA

3.1 Introduction

With the assistance of Josiah Oyalo (University of Eldoret), Moses Kosgei (Egerton University), the Food Processing Training and Incubation Centre (FPTIC) managed by Prof. Violet Mugalavai (University of Eldoret), the Kenya Livestock Agriculture Research Organization (KALRO), and the Uasin Gishu County Government, two field trials were conducted to examine the extent of aflatoxin contamination in maize in Uasin Gishu County, Kenya. These trials serve as a case study which seeks to inform researchers and governments about the state of aflatoxin contamination in Western Kenya. Maize samples were collected from across the county during the months of October (Trial 1) and December (Trial 2) 2019. The objective of the case studies was to determine: (1) Where in the agricultural supply chain aflatoxin contamination is the greatest risk; (2) Which (if any) particular areas of Uasin Gishu experience higher rates of aflatoxin contamination and (3) Derive recommendations for the appropriateness of extrusion technology as a means to decontaminate aflatoxin-contaminated maize within the complex food supply chain that exists in developing countries like Kenya.

3.2 A Case Study in Uasin Gishu County

Uasin Gishu County, located in Western Kenya's Rift Valley Province, is affectionately known among Kenyans as the country's "bread basket" because of its large annual maize production (World Bank Group, KCSAP, & Republic of Kenya, 2017) . For the 2019 harvest alone, the national government estimated that Uasin Gishu and Trans Nzoia counties would contribute 32 million bags (2.88 million metric tons) of maize to the country's national supply (Ndanyi, 2019). Since a majority of Kenya's population relies on high-maize producing counties for food security, Uasin Gishu was identified as a viable county to conduct research on aflatoxin contamination in maize. Since maize from Uasin Gishu is shipped throughout Kenya, understanding the extent of aflatoxin contamination there is critical for ensuring public health and food safety for the country as a whole.

Uasin Gishu County, located in Western Kenya's Rift Valley Province as seen in Figure 3.1 has a population of 894,611 people, which is predicted to surpass 1.3 million people by 2030 (NCPD, 2017). As seen in Table 1, the county is divided into six sub-counties (also known as Constituencies), which are: Soy, Kesses, Turbo, Kapseret, Moiben, and Ainabkoi. Each sub-county is further divided into wards depending on size, ranging from seven wards in the largest county (Soy) to three wards in the smallest county (Ainabkoi). The wards belonging to each sub-county in Uasin Gishu are also displayed in Table 1. Sub-counties converge in Eldoret Town, a city located north-west of the county's center. Given Uasin Gishu's large production of maize and high rainfall (1000 - 1250mm annually) maize crops are inevitably predisposed to aflatoxin infection (World Bank Group et al., 2017).

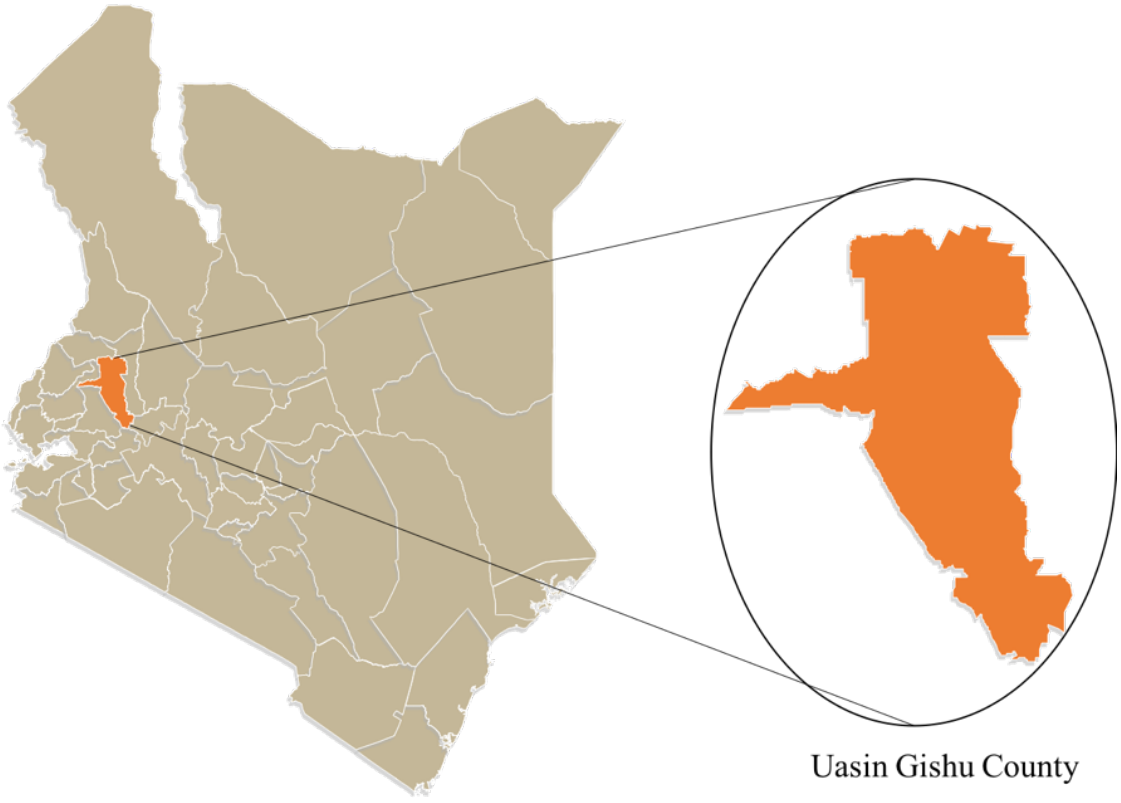

Figure 3.1: Map of Kenya. Uasin Gishu county (orange) is located in Western Kenya in the Rift Valley Province. Modified from Kenya Political County Template (yourfreetemplates.com, n.d.)

Table 3.1: Distribution of wards and sub-counties in Uasin Gishu County.

Sub-County	Ward Names	Number of Wards
Ainabkoi	Ainabkoi/Olare, Kapsebet, Kapsoya	3
Kapseret	Kipkenyo, Langas, Megun, Ngeria, Simat/Kapseret	5
Kesses	Cheptiret/Kipchamo, Racecourse, Tarakwa, Tulwet/Chuiyat	4
Moiben	Karuna/Melbeki, Kimumu, Moiben, Sergoit, Tembelio	5
Soy	Kapkures, Kipsomba, Kuinet/Kapsuswa, Moi's Bridge, Soy, Ziwa	7
Turbo	Huruma, Kamagut, Kapsaos, Kiplombe, Ngenyilel, Tapsagoi	6
	Total Number of Wards in Uasin Gishu	*30*

3.3 Aflatoxin in Maize Products: Trial 1

3.3.1 Materials and Methods

To determine the extent of aflatoxin contamination in Eldoret, 100 goro-goro samples of maize were purchased from all six sub-counties within Uasin Gishu County. A goro-goro is a traditional Kenyan unit of measurement used in the agriculture and food sector. One goro-goro is equivalent to approximately 2 kilograms. Note that economic conditions can effect goro-goro quantities, i.e. lower maize prices result in lesser volume per goro-goro and vice versa. Samples were bought and collected from three different types of distributers: farmers, traders, and poshomoris. Poshomoris are small-scale commercial millers that sell whole maize and ground maize flour. Only whole maize samples were purchased. Traders were considered suppliers who did not own their own milling machine and sold maize (usually along with other agricultural goods) in a market or road side stand. The term "trader" is sometimes used interchangeably with market vendors. Farmers were identified as individuals who considered farming to be their primary occupation. Samples were bought from small-scale (<5 acres), medium-scale (6-20 acres), and large-scale (>20 acres) farmers to understand if wealth (as demonstrated by land ownership)

correlated with a farmer's ability to mitigate aflatoxin contamination. Samples were collected from farmers with the help of the Uasin Gishu Directorate of Agriculture Extension Officers.

To ensure an accurate representation of Uasin Gishu County in entirety, at least three samples were collected from each ward for a minimum of 90 total samples. The origin, harvest month, storage type, moisture content, vendor type, drying method, and pesticide use was recorded. A target of 50% was set for the number of samples collected from farmers while the remaining samples came from market vendors (traders) and posho mills. For farmers, the number of acres owned was also recorded to assist in an analysis of small-holder versus medium and large sized farm owners in their ability to mitigate aflatoxin contamination levels. Samples were collected in paper bags and numbered. For any future researcher conducting field work in Kenya, it should be noted that plastic bags (including Ziplocs) are illegal in Kenya and are unable to be purchased even for research purposes. Samples were tested for moisture content (MC) (%wb) using a portable moisture meter (agraTronix, MT-16, Streetsboro, OH, USA) within 48 hours of collection. Moisture content was measured in triplicate to determine the average moisture content in the sample. Samples were stored under cool and dry conditions until aflatoxin testing (~2 weeks).

3.3.2 Aflatoxin analysis

All maize samples were cleaned, milled (at least 50% passes through a 20 mesh screen), and analyzed in duplicate for aflatoxin levels using the Helica Total Aflatoxin Kit (Helica Biosystems Inc., CAT. NO. 941AFL01M-96). Total aflatoxins (B1, B2, G1, G2) were tested for utilizing a rapid, quantitative, competitive enzyme-linked immunoassay (Helica Biosystems Inc., CAT. NO. 941AFL01M-96). All materials, including reagents, were refrigerated between 5 – 8 °C until testing. When preparing for aflatoxin testing, all kit materials were brought to room temperature (~25 °C) per instructions from the supplier.

Extraction Procedure

1. 2000 mL (2 L) of Extraction Solution composed of 70% methanol, 30% deionized water was prepared in a large glass laboratory bottle.
2. A random, representative sample of milled maize flour (~100- 200g) is selected.

3. 10 g of the sample is weighed and added to 50 ml of the Extraction Solution in a 125 ml air-tight container with a sealable lid. Note: In the HELICA Total Aflatoxin procedure, 100 ml of Extraction Solution is added to 20 g of sample in a 1:5 sample to Extraction Solution ratio. For this research, the sample size was scaled down for ease of testing and per recommendation of local food safety officials.
4. The sample is shaken by hand for 2-3 minutes (a blender can also be used, but was not available due to equipment access during this study).
5. All the particulate matter in the sample is allowed to settle. Then, using Wattman #1 filter paper, the liquid extract is separated from the solid particulates. 5 – 10 mL of sample is collected and the sample is now ready for the assay procedure.

Assay Procedure

The Helica Total Aflatoxin Assay is a solid phase direct competitive enzyme immunoassay. For this study, tests were conducted in duplicate for more statistical significance. The following steps are used to conduct the assay procedure:

1. Ensure that all reagents are brought to room temperature before beginning the assay procedure.
2. Place one Dilution Well in a microwell holder for each Standard and Sample to be tested. For this study, twelve samples were tested at a time because the microplate reader utilized could only run twelve samples at a time. Prepare an equal number of Antibody Coated Microtiter Wells in another microwell holder.
3. Using a micropipette, dispense 200 µL of the Conjugate into each Dilution Well. It is not necessary at this time to change the micropipette tip since there is no cross contamination possible at this step.
4. Next, using a new pipette tip for each microwell, add 100 µL of each Standard and Sample to the appropriate Dilution Well containing conjugate. Mix by priming the pipettor at least three times. Note that the operator must be aware of each Sample's location in the microwell holder at this time. When conducting a large number of tests (such as 100 for this study) it is easy to lose place of which specific microwell corresponds with which sample. The operator should take care to keep track of each Sample.

5. Using a new pipette tip for each Sample, transfer 100 μL of contents from each Dilution Well to the corresponding Antibody Coated Microtiter Well. Incubate the Samples at room temperature for 15 minutes.
6. Decant the contents from the Antibody Coated Microtiter Wells into a discard basin. Wash the microwells by filling each with distilled or deionized water, then decant the water into a discard basin. Repeat the wash for a total of 5 washes. Note that for this study, deionized water was utilized.
7. Tap the microwells (face down) on a layer of absorbent towels to remove the residual water.
8. Measure the required volume of Substrate Reagent (1 ml/strip or 120 μL/well) and place in a separate container. Add 100 μL to each microwell. Incubate at room temperature for five minutes. At this point, the samples will become blue in color. Note that aflatoxin contamination is inversely proportional to optical density (i.e. wells that exhibit a less-blue color have a higher aflatoxin contamination level).
9. Measure the required volume of Stop Solution (1 ml/strip or 120 μL/well) and place in a separate container. Add 100 μL in the same sequence and at the same pace as the Substrate was added. At this point, the contents of the microwell should turn some variation of yellow (except for those high in aflatoxin, which will remain clear).
10. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450 nm filter. Record the optical density of each microwell. Note that for this study, the microplate reader automatically created a standard curve for the tests and reported the aflatoxin level in ppb. The microplate reader automatically creates a standard curve which can be seen in Figure 3.2.

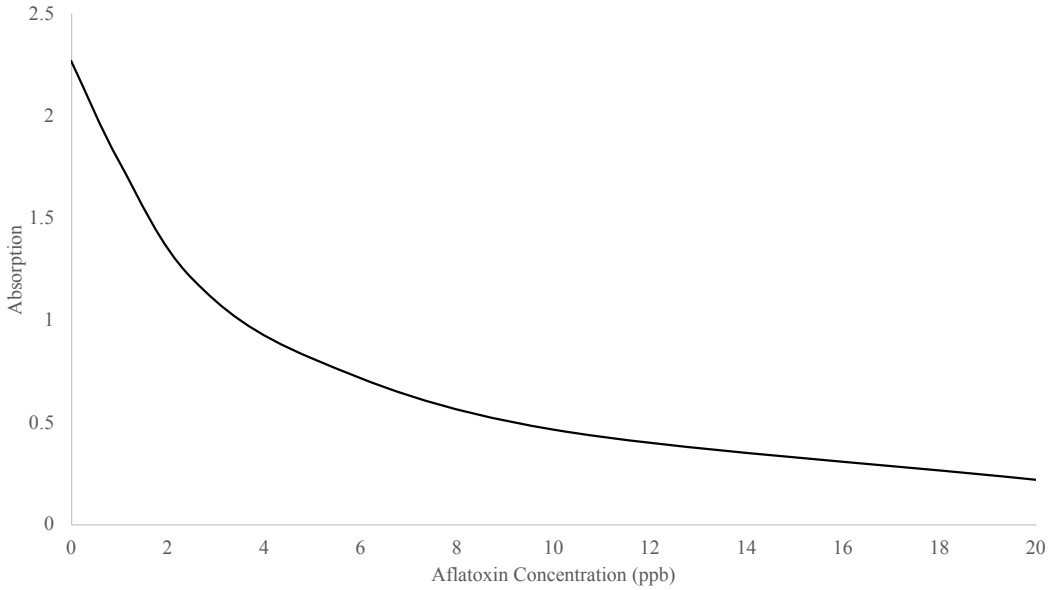

Figure 3.2: Standard curve for aflatoxin tests

3.3.3 Data Analysis

Descriptive statistics were used to identify trends from the collected data. Additionally, a multiple linear regression was conducted to understand the relationship between aflatoxin levels and other critical parameters as identified in aflatoxin literature and through field work observations. Dummy variables were used where there were categorical variables. A Type II ANOVA analysis was used to determine the significance of model parameters. All parameters were initially included in the regression analysis and insignificant variables were sequentially removed using a significance threshold of 0.5 until no p-values remaining were above the given threshold. Some variables, such as product source and farm size, were removed from the model due to lack of consistent data and a large variability. A series of scatter plots were also used to compare all variables and supplement the regression analysis. Appendix A includes all scatter plots generated comparing all possible combinations from the collected field data. The data was analyzed in R Version 4.0.1 and the complete data set from Trials 1 and 2 can be found in Appendix B.

3.3.4 Results and Discussion

100 samples were collected from all six sub-counties in Uasin Gishu as seen in Figure 3.3. 49% of samples were collected from farmers while the remaining 51% came from Poshomoris and traders as seen in Figure 3.4. 83% of samples were stored using non-hermetic methods including unsealed nylon bags. Two samples were collected from completely open storage piles inside large storage rooms, where the maize was exposed to air and water. An inspection of these samples led to the discovery of pesticide residues, which likely kept the maize from being harmed by insects. However, direct use of pesticide chemicals on maize products is restricted in Kenya and largely inadvisable, due to its significant correlation with sickness and cancer rates.

The average time between sample collection and harvest was approximately six months, but a majority (56%) of samples were collected more than three months after harvesting. It is important to note that Uasin Gishu is a large county and during this study different parts of the county had already begun to harvest the 2019 crop. Thus, the recorded time between harvest date and sample collection date ranges from little as a few weeks up to one year. Recording the time between harvesting and sample collection is important for understanding if there is any relationship between length of storage and aflatoxin levels.

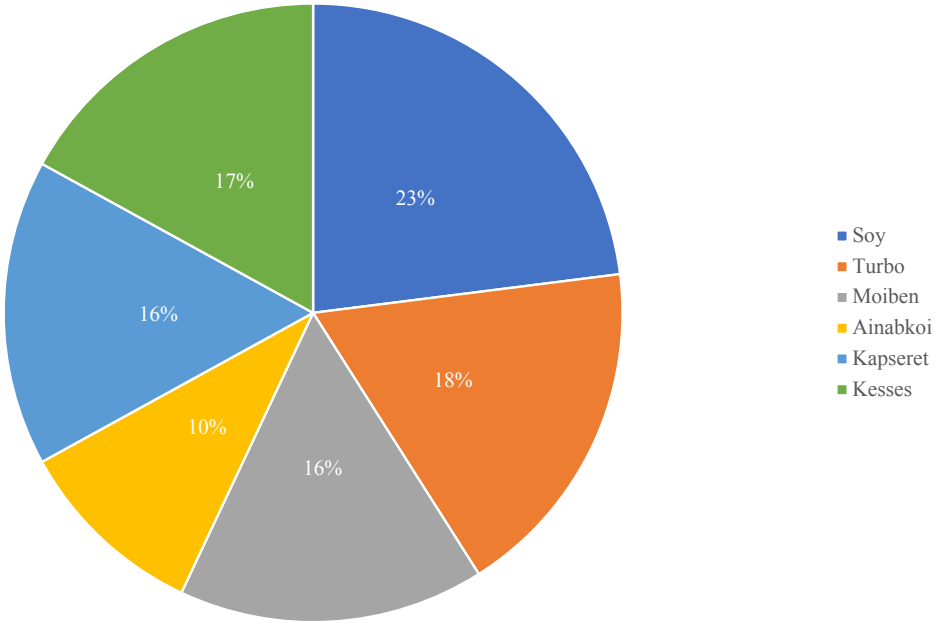

Figure 3.3: Distribution of maize samples collected within Uasin Gishu county.

Maize samples had an average moisture content of 13.3 %wb. The average moisture content recommended for optimized food safety in maize kernels is between 10 and 13% (Trenk & Hartman, 1970). Since all samples were dried on a tarp and likely without access to any standardized moisture meter, this average moisture content indicates that vendors may be improving their ability to determine if maize is dry. As expected, there was a positive correlation between grain moisture content and aflatoxin levels, but the correlation was not significant ($R^2 < 0.8$). Some samples had moisture content as high as 21.7 %wb even after drying. For future studies it would be valuable to test the pre and post milling moisture content. The moisture meter used in this study utilizes relative density from the surface of the grain to determine total moisture. Since drying methods are non-standardized, maize may not be evenly dried resulting in higher moisture content levels and corresponding aflatoxin levels inside the grain, which are more difficult to detect and destroy. Improving moisture content measurements would be of benefit to determining if there is any true correlation between high moisture content and aflatoxin levels. Additionally, more studies understanding how vendors – particularly farmers – determine the moisture content in their maize would be helpful to improve drying methods.

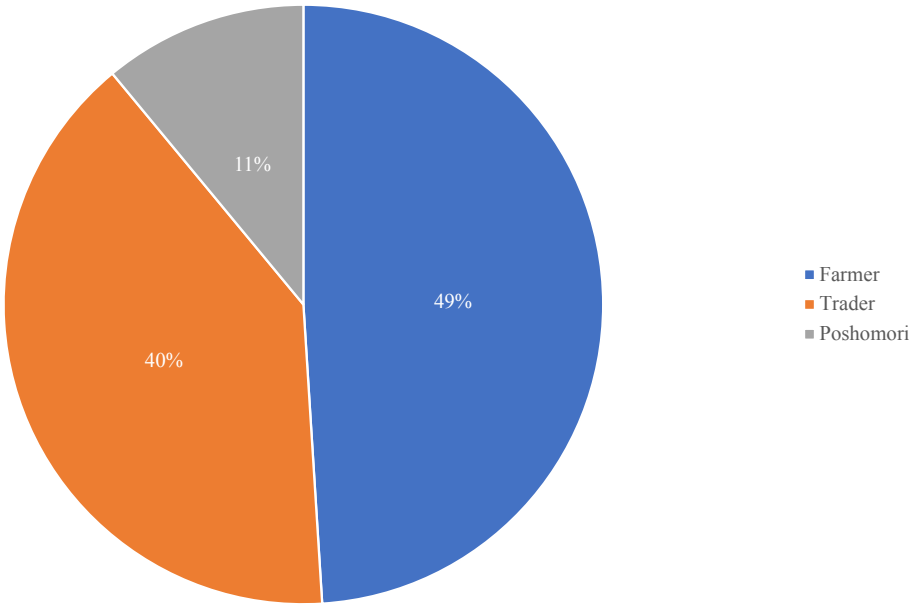

Figure 3.4: Distribution of vendor types from maize sample collection.

Only 5% of samples tested positive for aflatoxin levels >10 ppb, the tolerance level for safe human consumption designated by the Kenya Bureau of Standards (KEBS). This number is

notably lower than the 15% determined in a comprehensive study of aflatoxin in maize from Western Kenya in 2015 (Mutiga, Hoffmann, Harvey, Milgroom, & Nelson, 2015). Of those 5% of samples, 60% had aflatoxin levels greater than 20 ppb, rendering them unsafe for consumption by livestock as well.

A stepwise multiple linear regression was conducted to compare aflatoxin levels to all recorded variables. Non-significant variables were removed successively until there were no p-values greater than 0.5. The resulting model and its corresponding coefficients can be seen in Table 3.2. Only Storage Length was determined to be significant (p-value < 0.05) when controlling Constituency and Vendor type. It is important to note that the high number of samples testing for low levels of aflatoxin and the outlier-like behavior of samples that did contain high levels of aflatoxin, make any correlation generated biased towards predicting low aflatoxin levels and ultimately, any model is a poor predicter of high (> 10 ppb) aflatoxin levels. Additionally, while some variables are significant, the low R^2 value (0.1454) indicates that the model struggles to predict aflatoxin levels and there is likely a large confluence of factors effecting the results of this data.

However, results from the linear regression point to the general effects each variable may have on final aflatoxin levels. Moisture content has a positive effect on the final values indicating that a positive increase in moisture leads to greater amounts of aflatoxin. This follows with similar results found in studies comparing aflatoxin levels with moisture content (Trenk & Hartman, 1970). It is interesting to note that the effect of poshomori vendors on aflatoxin levels is negative. This negative effect on the final aflatoxin level may be indicative of poshomori's enhanced ability to ensure maize quality in comparison to other vendor types. Since poshomoris have the financial means to own mills, they may also have access to other resources that allow them to effectively dry maize or other mold mitigation technologies.

Interestingly, as storage length increases aflatoxin levels decrease. These findings contradict the general assumption that the longer a product is stored, the more likely it is to produce mold and aflatoxin as a results. However, further analysis of the data and field work methods indicate why such a negative trend is possible. First, aflatoxin is a secondary metabolite produced by mold. Often times, such mold is visible on the crop and grain that becomes moldy during storage is likely either discarded or fed to livestock. Thus, any grain stored for a long period of time (>10 months) and still used for human consumption is likely of higher quality, at least upon visible

examination. Second, older samples collected during this study were as old as one year. For farmers in particular, the ability to store grain for a longer period of time could also be an indicator of greater wealth. A larger net worth in the form of land acreage and access to quality storage could thus relate to lower aflatoxin levels. Third, the sample collection method may have contributed to lower aflatoxin levels in stored grain as farmers may have either (1) provided grain that was not truly stored for the length of time specified; (2) provided only their highest quality grain as a means to prevent the assumption that they produced low quality grain; or (3) Sold the grain they knew was low quality at the beginning of the harvest season and kept the high quality grain for personal consumption and/or sale later in the season.

Another important factor to note is that the average farm size in Trial 1 was 42.8 acres. While some samples (44%) were collected from small-holder farmers (owning less than 10 acres of land), a majority were collected from medium and large holder farmers. The prevalence of samples from medium and large-holder farms could also have influenced the aflatoxin levels in the samples. Amount of land farmed can be considered a proxy for wealth (Lowder, Skoet, & Raney, 2016). Thus, medium and large-holder farmers could have access to more aflatoxin mitigation tools and other post-harvest loss technologies not traditionally available to small-holder farmers. Future work would benefit from focusing on small holder farmers and aflatoxin prevalence in their crops.

Despite these findings, the low prevalence of samples with unsafe levels of aflatoxin is surprising given the history of aflatoxin contamination in Kenya. Such low percentages of contaminated samples could indicate that extension efforts and other interventions have been successful in counties like Uasin Gishu. The climate and elevation of Uasin Gishu may also play a role. Aflatoxin is generally produced at moisture contents greater than 17.5 %wb and temperatures greater than 24 °C (Trenk & Hartman, 1970). The average temperature in Uasin Gishu rarely reaches above 24 °C due to high elevation. In contrast, 60% of the contaminated samples were purchased from traders who sourced their maize from outside of Uasin Gishu. These contaminated samples came from low-lying, humid regions West of Uasin Gishu, such as Kakamega and Bungoma counties where aflatoxin is known to be endemic (Ndisio, 2015). When maize is grown in humid conditions it is more likely to be infected. It follows that traders in Uasin Gishu – including large grain processing companies – should be particularly cautious when sourcing maize and other aflatoxin-susceptible foods from regions outside the county.

It is possible that the timing of the study and the sampling method also introduced bias that resulted in such low levels of aflatoxin contamination. Since samples were collected with the help of extension officers, it is possible that extension officers chose farmers who they know adhere to best practices for aflatoxin mitigation. Choosing farmers who conduct best practices not only results in fewer contaminated samples, but also protects the extension officers reputation by proving that they are effectively doing their job. Additionally, farmers may only provide their highest-quality maize for a sample, also contributing to the bias in the study. Of the 5% of samples that tested for levels above 10 ppb, only one came from small-holder farmer (≤ 10 acres). However, it should be noted that aflatoxin is not always visibly quantifiable. Additionally, the heterogeneity of aflatoxin levels means that samples averages – even in samples as small as 0.5 kg – may be an inaccurate representation of the true aflatoxin levels present in the product.

3.3.5 Recommendations

Based on these findings, consumers and processors are cautioned against purchasing maize that comes from areas that experience high rainfall, relative humidity, and temperatures. Locally produced and processed maize from Uasin Gishu is possibly safe for consumption given the region's climate, but more holistic testing is necessary to confirm the safety of maize with certainty. Consumers should take note that aflatoxin levels in maize are highly dependent on agricultural conditions and practices, which change yearly and vary between producers. Additionally, with the heterogeneity of aflatoxin contamination in maize, it remains possible that the true extent of contamination is unknown and very difficult to predict. The cost barrier of aflatoxin testing (~5 USD/test) makes it difficult for researchers, government bodies, and private industry to test for aflatoxin in a country like Kenya, which lacks significant financial resources for this type of testing.

Table 3.2: ANOVA analysis of multiple linear regression model for Trial 1 data.

ANOVA				
	Sum of Squares	**Degrees of Freedom**	**F-Value**	**Pr (>F)**
Storage Length	125.93	1	7.2852	0.008288**
Vendor	83.5	2	2.4153	0.095049
Constituency	153.84	5	1.7799	0.124818
Residuals	1573.05	91		
Total	1936.32	99		

Model Summary		
R Square	**Adjusted R Square**	**Std. Error of the Estimate**
0.1454	0.07027	4.158

Coefficients				
	Beta Estimate	**Std. Error**	**T value**	**Pr (>|t|)**
(Intercept)	3.26294	0.80575	4.05	0.000108***
Storage Length	-0.27723	0.10271	-2.699	0.008288**
Farmer	1.28731	0.78868	1.632	0.106089
Poshomori	-2.17627	0.99874	-2.179	0.031913*
Ainabkoi	-0.92102	1.25064	-0.736	0.463358
Kapseret	2.01732	1.0459	1.929	0.056874.
Kessess	1.49182	1.00392	1.486	0.140739
Moiben	-0.57868	1.04342	-0.555	0.580528
Soy	-0.03699	0.87583	-0.042	0.966401

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

3.4 Aflatoxin in Maize Products: Trial 2

3.4.1 Justification for Trial 2

Inevitably when conducting research abroad, particularly in developing countries, there are unexpected hurdles that can affect research results. In particular, it is important to note that certain cultural and societal differences can influence field work results in the form of unrecognized bias.

Given the low number of samples testing positive for aflatoxin levels above 10 ppb, the field work methods for Trial 1 were re-evaluated. Upon re-evaluation, several biases in the research were identified. First, extension officers were utilized to help identify farmers who would be willing to participate in the study. It is likely that extension workers, whether consciously or not, chose farmers with whom they had a robust working and/or personal relationship. While this allowed for ease of sample collection, it also introduced bias in the form of pseudo-favoritism and eliminated randomness from the sample collection process. Second, the sample collection team was made up of two Kenyan males and one white, American female (the author of this thesis). The presence of a white, American during field work may have encouraged famers to give their best grain for fear of presenting a foreigner with a low-quality product.

The aforementioned bias factors provided good reasoning to conduct a second trial. In Trial 2, a Kenyan agricultural business graduate was hired to travel throughout the county and collect samples rather than the three-person team in Trial 1. It should be noted that this student also identified in the same tribal group (Kalangen) as most Kenyans working in the agricultural sector of Uasin Gishu County. Kenya is an extremely diverse country with more than 40 distinct ethnic groups. Having a researcher who shares the same ethnic identity as the farmers from whom samples were collected fosters trust in the research itself. For Trial 2, extension officers were no longer utilized by the research team to identify sample collection points (e.g. farmers), but the county agricultural office was still informed and updated on all research. Finally, samples collected from farmers were collected from those owning less than 10 acres. Given the correlation between land ownership and wealth, the number of acres owned by a farmer may indicate whether he or she has access to aflatoxin prevention methods. In Trial 1, working with extension officers frequently led to sample collection from large, wealthy farmers. This may also have influenced the final aflatoxin levels.

3.4.2 Materials and methods

Except for those differences outlined in section 3.3.5 regarding the elimination of bias for Trial 2, all materials and methods were conducted in the same fashion as Trial 1. For each sample, the origin, harvest month, storage type, moisture content, vendor type, drying method, pesticide use, and number of acres owned (for farmers only) was recorded. Paper bags were utilized to

collect samples and samples were evaluated for moisture content levels before milling. The post-milling moisture content was also analyzed because inefficient drying methods (e.g. tarp drying) often leave maize kernels with a high moisture content inside even though the outside is dry. The type of moisture reader utilized relies on relative density to measure moisture content on the surface of the grain, thus leaving the true internal moisture content unknown. Measuring the before and after milling moisture content allows one to see the true accuracy of moisture content measurement and effectiveness of drying. After milling, each sample was randomly tested in duplicate for aflatoxin using the same Helica Total Aflatoxin Assay Kit and procedure as seen in Section 3.3.2.

The same methods used to analyze Trial 1 were used to analyze Trial 2 results. Descriptive statistics were used to identify critical information from the collected data. Additionally, multiple linear regression was conducted to understand the relationship between aflatoxin levels and other critical parameters as identified in aflatoxin literature and through field work observations. The indicators include vendor type (farmer, poshomori, or trader), storage method (hermetic or non-hermetic), moisture content, and length of storage. Additionally, a model comparing aflatoxin levels with their location (i.e. sub-county of origin) was also studied to identify potential areas of concern for the Uasin Gishu government.

3.4.3 Results and Discussion

For Trial 2, another 100 samples were collected from all six sub-counties in Uasin Gishu as seen in Figure 3.6. 57% of samples were collected from farmers while the remaining 43% came from Poshomoris and traders. 89% of samples were stored using non-hermetic methods including unsealed nylon bags. One sample was collected from a completely open storage pile inside a large storage room, where the maize was exposed to air and water. As with Trial 1, an inspection of this sample led to the discovery of pesticide residue, which likely kept the maize from being harmed by insects. However, direct use of pesticide chemicals on maize products is restricted in Kenya and largely inadvisable, due to its significant correlation with sickness and cancer rates.

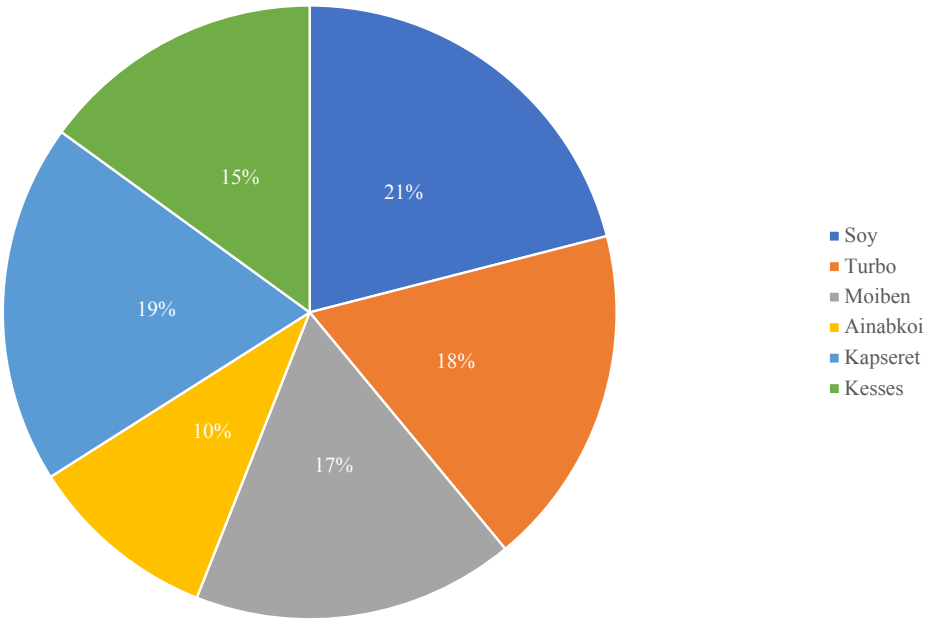

Figure 3.5: Distribution of samples collected from across Uasin Gishu county for Trial 2

The average time between sample collection and harvest was slightly less than one month. Given that Trial 2 was conducted during December 2019, when most of Uasin Gishu county is harvesting maize, such a short storage time logically follows the seasonal trends for food production in this region. It is important to note that such a short time between sample collection date and harvest time meant that some samples had not been completely dried by the time field work was conducted for Trial 2. As such, the average moisture content for these samples was greater than those collected in Trial 1. It should also be noted that Western Kenya experienced

unprecedented amounts of rain during the course of Trial 2. While Uasin Gishu county usually averages 1000 mm of rain during September, an unprecedented amount of rain fell during peak harvest time (November/December) during 2019 (World Bank Group et al., 2017)

Maize samples from Trial 2 had an average moisture content of 16.1 %wb. The average moisture content recommended for optimized food safety in maize kernels is between 10 and 13%. Since some samples were only briefly dried on a tarp before collection, this is likely why the average moisture content is higher than recommended levels. As expected, there was a positive correlation between grain moisture content and aflatoxin levels, but the correlation was not significant ($R^2 < 0.8$). For this trial, the moisture content of each sample was also measured after milling. The average moisture content post milling was 18.3 %wb, which is slightly higher than the average pre-milled moisture content. This difference in moisture content is indicative that conventional drying methods lack the ability to thoroughly dry the grain, which could leave the center of the grain still vulnerable to mold growth and aflatoxin contamination. However, as noted in the Results and Discussion section for Trial 1, relative humidity plays a role in aflatoxin contamination. Thus, while some samples in this study had moisture content levels as high as 29.6 %wb, a moderate climate and high elevation may prevent mold in the sample from producing aflatoxins.

Only 5% of samples in Trial 2 tested positive for aflatoxin levels >10 ppb, the tolerance level for safe human consumption designated by the Kenya Bureau of Standards (KEBS). This number is notably lower than the 15% determined in a comprehensive study of aflatoxin in maize from Western Kenya in 2015 (Mutiga et al., 2015). Of those 5% of samples, 100% had aflatoxin levels greater than 20 ppb, rendering them unsafe for consumption by livestock and humans. A conducted stepwise multiple linear regression found that no variables had a significant impact on aflatoxin levels in maize samples from Trial 2.

3.4.4 Recommendations

As with Trial 1, the low prevalence of samples in Trial 2 with unsafe levels of aflatoxin is surprising given the history of aflatoxin contamination in Kenya. Such low percentages of contaminated samples could indicate that extension efforts and other interventions have been proved successful in counties like Uasin Gishu. The climate and elevation of Uasin Gishu may

also play a role, since the county's higher elevation often correlates with lower humidity despite significant rainfall. At least 40% of contaminated samples were purchased from traders who sourced their maize outside of Uasin Gishu. However, the few samples testing positive for unsafe levels of aflatoxin, lack of reliable sourcing data, and heterogeneity of aflatoxin infection make such fixed conclusions undependable. Still consumers and processors should continue to be cautious when purchasing and consuming maize to avoid aflatoxin ingestion, particularly when the product is sourced from areas known for conditions that promote aflatoxin production, such as regions with high temperatures, rainfall, and humidity.

3.5 Comparison of Aflatoxin Trials 1 and 2

It is interesting to note that both Trial 1 and Trial 2 had only 5% of samples test positive for unsafe levels of aflatoxin. This supports the idea that Uasin Gishu county may be more effectively addressing challenges with aflatoxin than neighboring counties. These same results also confirm that the potential bias identified in Trial 1 had little to no effect on the results, since the same results were achieved during Trial 2. However, similar studies with larger sample collection sizes would be needed to confirm such a conclusion. Additionally, cost barriers prevented the entire maize sample from being test. A more representative aflatoxin level could be determined by testing the entire sample rather than random 20 gram selections.

When combining the data from Trial 1 and Trial 2 and running a stepwise multiple linear regression, storage length and constituency remained were both significant when controlling the Trial Number, Storage type, and the Vendor type. Similar to Trial 1 analysis, storage length has a negative effect on aflatoxin levels. That is, as storage length increases, aflatoxin levels decrease. This finding is immediately counterintuitive, but upon further analysis aligns with the conditions under which field work was conducted. In particular, Trial 2 was conducted during the 2019 harvest season (Nov – Dec) which experienced unusually high levels of rainfall in comparison to previous years. Thus, samples collected 1 – 2 months after harvesting were generally harvested recently and under less ideal agricultural conditions than the previous year's (> 10 months storage length). Thus, the negative correlation between storage length and aflatoxin levels aligns with this finding.

Table 3.3: ANOVA analysis of multiple linear regression model for Trial 1 and Trial 2 data.

ANOVA				
	Sum of Squares	**Degrees of Freedom**	**F-Value**	**Pr (>F)**
Storage Length	114.35	1	5.9972	0.01552
Vendor	81.6	2	2.1397	0.1214
Constituency	223.3	6	1.9518	0.07635
Storage Type	15.91	1	0.8343	0.36254
Residuals	2764.85	145		
Total	3200.01	155		

Model Summary		
R Square	**Adjusted R Square**	**Std. Error of the Estimate**
0.1132	0.05248	4.354

Coefficients				
	Beta Estimate	**Std. Error**	**T value**	**Pr (>|t|)**
(Intercept)	4.8914	1.8	2.717	0.00737**
Storage Length	-0.2493	0.1022	-2.439	0.01592*
Farmer	0.8075	0.744	1.085	0.27955
Poshomori	-2.0822	1.0038	-2.074	0.03981
Ainabkoi	-1.2436	1.1532	-1.078	0.28264
Kapseret	2.4029	0.8063	2.98	0.00337**
Kessess	0.5208	0.8551	0.609	0.54345
Moiben	-0.5037	0.9066	-0.556	0.57936
Soy	0.2058	0.7056	0.292	0.77097
Trial	-1.4709	1.1313	-1.3	0.19558

*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

3.6 Conclusion

200 maize samples from across the county of Uasin Gishu were collected and analyzed for aflatoxin levels for this study. In Trial 1 and Trial 2, only 5% of samples had unsafe levels of aflatoxin (> 10 ppb). While these low percentages are promising indicators for the effectiveness of aflatoxin mitigation and education in Uasin Gishu, caution should still be taken when working with such a highly-carcinogenic toxin. Additionally, given Kenya's history of widespread aflatoxin contamination in maize, larger studies (>100 samples) with ample resources for aflatoxin testing are necessary to fully understand the extent of contamination in any region. Still, given the low rates of contamination demonstrated in this study, Uasin Gishu may serve as a viable control region for future aflatoxin mitigation studies in Kenya or in East Africa at large.

Storage length was a significant variable in understanding aflatoxin levels in maize samples from across the county. Longer lengths of storage correlated with lower levels in aflatoxin, which is initially counterintuitive given the understanding of crop storage and post-harvest loss. However, when evaluating length of storage as a proxy for net wealth – especially among farmers – it aligns well with the results that farmers with larger amounts of land and thus more stored maize, are able better able to afford aflatoxin mitigation technologies and practices. However, storage length is highly dependent on other variables such as vendor type, study bias, and storage type. This dependency on other variables is indicated by a very weak ($R^2 << 0.8$) correlation between storage length and aflatoxin levels in the collected samples. Additionally, the agricultural conditions under which this field work was conducted included particularly high rainfall and thus, samples collected closer to their harvest date were more subject to high levels of moisture and poor growing conditions, which promote aflatoxin production in maize.

From this work, Uasin Gishu was identified as a county that may struggle less with aflatoxin contamination in maize than surrounding regions lower elevation, higher temperatures, and more humid conditions. Consumers and processors are cautioned against purchasing maize that comes from lower-elevation, high-temperature, and high-rainfall conditions. Consumers should take note that aflatoxin levels in maize are highly dependent on agricultural conditions and production practices, which change yearly and among producers. Farmers are encouraged to continue good agriculture practices, including the recommendations by Uasin Gishu county extension to dry maize to a level between 10 and 13.5% moisture content. This study lacked the

funds necessary for holistic testing and, given the inherent heterogeneity of aflatoxin contamination, leaves the true extent of contamination largely unknown and very difficult to predict. Thus, given the toxicity of aflatoxin and its danger to consumers, producers and processers should continue to take all necessary precautions to prevent aflatoxins irrespective of source since it is impossible to say that maize from any region is safer than another without further testing.

Finally, it is important to note the role of bias in this study, considering that a majority of the work was conducted from the standpoint of a white, American female working in Kenya. A standpoint should be viewed "not [as] a given and finalized form of knowledge, but as a ground in experience from which discoveries be made" (Ngo, 2013; Smith, 2005). As such, factors like social justice, intersectionality, and intercultural competence - which go beyond technical expertise - can dramatically influence the success of international projects such as this one. In particular, it is important to note that certain cultural and societal differences can influence field work results in the form of unrecognized predispositions. Such bias, which is exacerbated by a researcher's lack of local knowledge, policies, and cultural norms, can impede the efficiency, complexity, and validity of field research.

4. OPERATIONAL EXPERIENCES WITH UTILIZING SMALL-SCALE EXTRUSION TO DEGRADE AFLATOXIN IN CONTAMINATED MAIZE

4.1 Introduction

Understanding the ability of small-scale extrusion to decontaminate aflatoxin infected foods is critical to the feasibility of utilizing such food processing technology in the developing-country context. Such technology must be affordable, well-designed, and effective in rendering contaminated foods safe for human and livestock consumption. To know the effects of small-scale extrusion on aflatoxin decontamination. a series of experiments were conducted using maize at varying moisture contents (35, 40, and 45 %wb) and motor frequencies (15, 38, and 50 hz). Samples were measured for both pre and post extrusion aflatoxin levels. Later, a 2^3 factorial design was used to analyze the resulting data. This analysis was used to determine the significance of each variable as well as their individual effects. Total percent degradation was then compared to maximum product temperature, feed rate, residence time, and moisture content. Finally, processing conditions for optimal aflatoxin degradation were determined.

4.2 Extruder Description

A Mini-Extruder™ (Technochem International, Inc., 967 Quartz Avenue, Boone, IA, 50036, USA) was used to conduct all extrusion experiments for this research. Mini-Extruder™ was co-developed by Purdue University (West Lafayette, IN) and Triple F. (Des Moines, IA) with funding from the National Aeronautics and Space Administration (NASA). The exact Mini-Extruder™ was purchased as part of the USAID Feed the Future Food Processing Innovation Lab project and is used to create a variety of food products using local ingredients at the University of Eldoret Food Process Training and Incubation Centre (FPTIC) in Eldoret, Kenya. The Mini-Extruder™ is processes approximately 60 pph (30 kg/hr) of product. Figure 4.1 shows a diagram of a small-scale extruder similar to the Mini-Extruder™. The only major difference between the two designs is the lack of sloped bushing in the Mini-Extruder™.

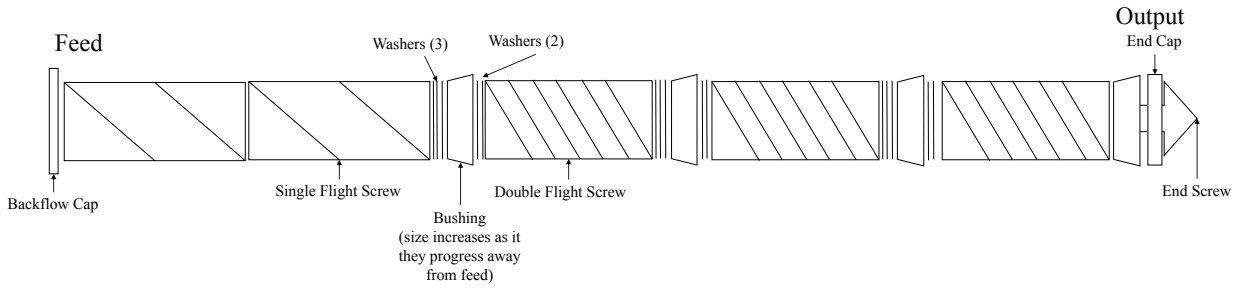

Figure 4.1: Diagram of small-scale extruder (60 pph). (Hauserperger, 2017).

4.3 Extruder Operating Procedure

The assembly procedure was adapted from the Technochem Mini-Extruder™ manual and used for all product testing. Special notes are included where necessary for certain product testing. Otherwise, all product testing utilized the same extruder operating procedure.

1. Assemble the extruder starting with inserting the key along the key way until it reaches the feed chamber bushing.
2. Insert the first feed-screw (single flight) along the key way, followed by the second single flight feed screw.
3. Insert the first shear bushing.
4. Insert the first double-flight screw along the key way.
5. Insert the second shear bushing followed by the second double-fight screw.
6. Insert the third shear bushing followed by the third double-flight screw.
7. Insert the fourth shear bushing.
8. Insert the double-flight push screw. By this point, the entire key shaft should be covered by screws and shear bushings. The end of the final double-flight push screw should be flush with the end of the key shaft.
9. Insert the bullet with washer head at the end of the key shaft and tighten anti-clockwise with a wrench turn. This final piece is tightened anti-clockwise to counter-act the clockwise spinning of the extruder screw, which would loosen the key if tightened in the clockwise direction.

10. Insert the feed chamber over screws until it locks with the dowels. The feed chamber must lock with the dowels to prevent unwanted spinning of the chamber. Make sure the Feed-Chute Receptor is facing up. Next pace bolts over the dowels on both sides of the chamber and tighten the bolts to hold the chamber in place.
11. Insert the first barrel over the screws until it is flush with the feed chamber. Secure the barrel by first placing the bottom clamp in position at the edge where the feed chamber and the first barrel meet. Note that the barrel a tapered edge which must match the the tapered side of the clamp. The feed barrel (part of the feed chamber) has a straight edge. The bottom clamp can be distinguished from the top clamp by its screwed hole. Place the top clamp into position over the bottom clamp and tighten using bolts. It is very important to ensure that clamps are properly tightened the barrel, so as to prevent unwanted slipping due to rotation of the screw and product flowing inside.
12. Place the second barrel over the screws until it is flush with the first barrel. To ensure that the second barrel is properly flush with the first, a hammer can be used to gently tap the barrels until they are fit snuggly. Repeat the clamping procedure described in Step 11.
13. Repeat step 12 with a third barrel.
14. Place the end cap in position at the end of the screw. Repeat the same clamping procedure described in Step 11, using a fourth clamp to secure End Cap to the Third Barrel.
15. Place the nose cone or oil expelling attachment to the End Cap and tighten the locking screw on the side of the End Cap to secure. Note that for the experiments described in this thesis a circular die 5 mm in diameter was used.
16. A thermometer hole is located on the top left corner of the end cap. A thermometer can be placed here to measure the die/outgoing feed temperature.
17. Place the Feed-Chute (also called a hopper) in position over the Feed Chamber (described in Step 10). Screw the Feed-Chute in place until. The mini-extruder has now been assembled.

4.4 Extrusion of Maize Flour

4.4.1 Materials and Methods

Aflatoxin-infected maize (>10 pbb) from a domestic contaminated shipment was purchased from Unga Factory Limited in Eldoret, Kenya. The contaminated maize was milled using a cone mill and stored under hermetic conditions at room temperature (20 – 25°C). The moisture of the milled maize was measured using a portable moisture meter (agraTronix MT-16 Grain Moisture Tester Part No. 08155), which is commonly utilized by extension officers in Kenya. Samples of 2 - 4 kg of contaminated maize were prepared at 30, 35, 40, and 45 %wb moisture content. Samples were stored in a refrigerator overnight (~ 8 - 12 hours) to allow the moisture content to equilibrate. It is important that the moistened samples are refrigerated during storage to prevent naturally occurring microbes from fermenting the maize during equilibration.

Upon preparation of samples for extrusion, the maize was brought to room temperature. Although the average aflatoxin level in the bags was provided (12.5 ppb), 14, 20 g samples per moisture content were removed and tested for aflatoxin using the method outlined in Section 3.3.2. to serve as pre-extrusion controls due to the well-known heterogeneity of aflatoxin contamination. The extruder was then prepared as described in Section 4.3. The moistened contaminated samples were tested at motor frequencies of 15, 38, and 50 hz. Samples for extrusion ranged from 0.1 – 0.2 kg in size. Maximum die temperature, ambient temperature, and feed rate were record for each sample. Feed rate was starve fed and determined by diving total mass versus processing time.

A 2^3 factorial design was used to study the influence of moisture content (% wb) and motor frequency (hz) on aflatoxin decontamination in maize products. Each factor has three levels. The statistical model for this design is seen in Equation 3.

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk} \quad \begin{cases} i = 1, 2, 3 \\ j = 1, 2, 3 \\ k = 1, 2, 3 \end{cases} \tag{3}$$

Where μ is the grand mean (% aflatoxin degradation), τ_i i^{th} effect level of factor A (moisture content) ignoring B, β_j j^{th} effect level of factor B (extruder speed) ignoring A, $(\tau\beta)_{ij}$ is the

interaction effect of moisture content and extruder speed, and ϵ_{ijk} is the random error. It is assumed that the model follows the following constraints:

$$\sum_i \tau_i = 0 \quad \sum_j \beta_j = 0 \quad \sum_i (\tau\beta)_{ij} = 0 \quad \sum_j (\tau\beta)_{ij} = 0 \tag{4}$$

QQplots were generated to test the data for normalcy. Next, using ANOVA, the significance of each factor was tested and the effects of each variable on aflatoxin degradation were analyzed. Finally, the optimal processing conditions for greatest percent aflatoxin degradation were determined. These optimal conditions were then used to determine the residence time under the optimal processing conditions for aflatoxin degradation inside the mini-Extruder™ (A. Kumar, Ganjyal, Jones, & Hanna, 2006). Total percent aflatoxin degradation was later compared to other processing conditions, including maximum product temperature and feed rate.

4.5 Results and Discussion

Maize extrudates were developed for each combination of moisture content (35, 40, 45 %wb) and motor frequency (15, 38, 50 hz). Raw data is available in Appendix C. There is a large standard deviation between replications for aflatoxin degradation, which is likely due to aflatoxin heterogeneity within a sample. Note that motor frequency is related to and serves as a proxy for screw speed. In general, higher motor frequencies results in a higher screw speed. This relationship further described and calculated in Appendix F. Samples tested below 35% moisture were excluded from any further testing because they frequently burned inside the extruder at low motor frequencies (e.g. 15 hz) and the resulting burnt product is obsolete and undesirable for almost any consumer.

For this study, the extruder was hand fed. It is possible that a more consistent feed rate would have prevented burning from occurring with low-moisture products. In some cases, it was helpful to run samples first at higher motor frequencies (50 hz) and then lower frequencies to ensure the extruder was brought to a higher temperature before running at lower shear rates. Previous studies had success running moisture contents below 35 %wb using similar methods (Penner, 2011). Penner recorded that when the screw speed was gradually reduced from 750 rpm to 450 rpm that the product did not clog in the extruder and burn. Burning is also the result of

increased shear forces produced by a combination of high screw speed and low moisture content. When moisture content is low, the product behaves more as a solid (e.g. increased viscosity) and is simultaneously exposed to higher temperatures, resulting in increased shear stress. To prevent burning, samples with low moisture content were processed at higher steady state feed rates (> 20 kg/hr). As a result, samples at 35, 40, and 45 % wb had average flow rates of 28.6, 22.9, and 4.9 kg/hr respectively.

The importance of moisture content during extrusion processing can also be seen by the maximum product temperature. Using a thermocouple, the maximum product temperature was recorded at the extruder die for each sample. It was assumed that the maximum temperature at the die was representative of overall maximum product temperature, creating a linear temperature profile for the product inside the extruder (Tonner, 2018). At high moisture content (45 %wb) samples failed to reach temperatures greater than 65 °C, while samples of lower moisture content (35 and 40 %wb) reached temperatures greater than 80 °C. The difference in temperature between the samples can again be attributed to moisture content and the resulting shear forces. Products with a lower moisture content experience larger shear forces as the result of increased viscosity and thus reach a higher maximum temperature inside the extruder. Products with high moisture content experience less shear forces due to increased lubrication and decreased viscosity. It is important to note the strong codependence that exists between shear, temperature, and viscosity as well as their inherent relationship to product moisture content.

Post-extrusion aflatoxin percent degradation, flow rate, maximum temperature, and the corresponding operating conditions can be found in Table 4.1. The total percent degradation ranged from 11.8 to 81.5% when comparing to the measured pre-extrusion aflatoxin levels at moisture contents between 35 and 45 %wb and motor frequencies varying from 15 to 50 hz. It was assumed that the measured pre-extrusion aflatoxin levels were more representative than the average aflatoxin values provided by the grain supplier, who utilized the USDA sampling standard for aflatoxin to test the contaminated maize. Often post-processing aflatoxin levels are compared to a large contaminated sample (e.g. 90 kg). However, the heterogeneity of aflatoxin lends itself to such measurements being largely inaccurate and non-representative of the true aflatoxin levels in the bag even after sufficient mixing. As such, the purchased 90 kg bag of maize was split into nine, 10 kg samples. One 10-kg bag was chosen for extrusion testing and the pre- aflatoxin levels were measured again immediately after splitting the 10 kg into three 2.5 kg samples and adjusting

the moisture content in each to 35, 40, and 45 %wb. The samples were measured for aflatoxin a minimum of 10 times for at each moisture content. The resulting pre-extrusion aflatoxin levels were 2.3, 5.8, and 8.7 ppb at 35, 40, and 45 %wb respectively. It was assumed that degradation occurred at the same rate regardless of starting aflatoxin concentrations.

Table 4.1: Results for aflatoxin percent degradation, maximum die temperature, and mass flow rate at 35, 40, and 45 %wb and 15, 38, and 50 hz.

Symbol	Motor Frequency (hz)	Moisture (%wb)	Maximum Temperature (°C)	Avg. Feed Rate (kg/hr)	Avg. % Degradation
▲	15	35	83.5	25.0	26.2
■	38		68.9	22.6	23.3
●	50		70.5	38.2	11.8
▲	15	40	86.6	21.3	65.8
■	38		83.9	17.4	70.1
●	50		64.7	29.9	69.2
▲	15	45	60.1	5.5	76.6
■	38		62.4	4.5	83.1
●	50		62.7	4.9	81.2

As previously mentioned, moisture content plays an important role in the maximum product temperature and feed rate during extrusion processing. The importance of moisture content is further reiterated upon statistical analysis of the aflatoxin data. Table 4.2 shows the results from conducting an analysis of variance (ANOVA) on the 2^3 experimental design with moisture content and motor frequency as independent variables and aflatoxin percent degradation as the dependent variable. The results of the ANOVA analysis indicate that the effect of moisture content was significant (p-value < 0.05), while motor frequency was not (p-value > 0.05). Moisture content was also found to be significant when extruding peanut meal and during other aflatoxin degradation processing methods (Liu et al., 2018; Wang et al., 2018; Zheng, Wei, Xu, & Fan, 2015). The combined effect of moisture content and motor frequency was also not significant (p-value > 0.05).

Using the ANOVA results from Table 4.2 and Excel's solver function, the optimal operating conditions for aflatoxin degradation were determined using a regression table as seen in Appendix D. The model created using the regression table has an R^2 of 0.89, indicating that predictions are reliable and significant. Using this method, maximum aflatoxin degradation occurs

at a combination of 43 %wb moisture content and 45 hz motor speed. These processing conditions align with the results seen in Figure 4.2, where individual effects of moisture content and motor speed on total percent aflatoxin degradation are shown. It is interesting to note that the worst aflatoxin degradation occurs at 35 %wb and 50 hz motor speed. Values for individual effects graphed in Figure 4.2 can be found with the regression analysis in Appendix D.

The impact of varying moisture content is reiterated when considering its impact on product residence time within the extruder. As previously mentioned, higher feed rates were necessary to prevent burning in low moisture products. As such, at lower moisture contents would likely correlate with decreased product residence times. To determine residence time at each processing condition, the linear relationship between moisture content and feed rate was used as seen in Figure 4.3. While the R^2 value is less than 0.8, knowledge from literature on rheology and moisture content during extrusion provides support for the assumption that residence time is closely related to moisture content (Bouvier & Campanella, 2014). Using the linear relationship seen in Figure 4.3, the feed rate at the maximum processing condition of 43 %wb can be roughly approximated at 11.7 kg/hr. Multiplying the feed rate by the residence time, the volume of product

Table 4.2: ANOVA analysis of extrusion data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	18126.93449	2265.86681	18.18	< 0.0001
Error	17	2118.92267	124.64186		
Corrected Total	35	20245.84615			

R-Square	Coeff Var	Root MSE	% Degradation Mean
0.895341	19.49182	11.16431	57.27692

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Moisture	2	17470.09726	8735.04863	70.8	<0.0001
Motor Frequency	2	130.72379	65.36189	0.52	0.6012
*Moisture*Motor Frequency*	4	526.11343	131.52836	1.06	0.4085

inside the extruder under steady state conditions is approximately 0.33 kg. Assuming that this is the same mass of product inside the extruder during any steady state processing the residence time can be calculated by dividing 0.33 kg by the average feed rate at each processing condition. The resulting residence times for each processing condition can be seen in Table 4.3.

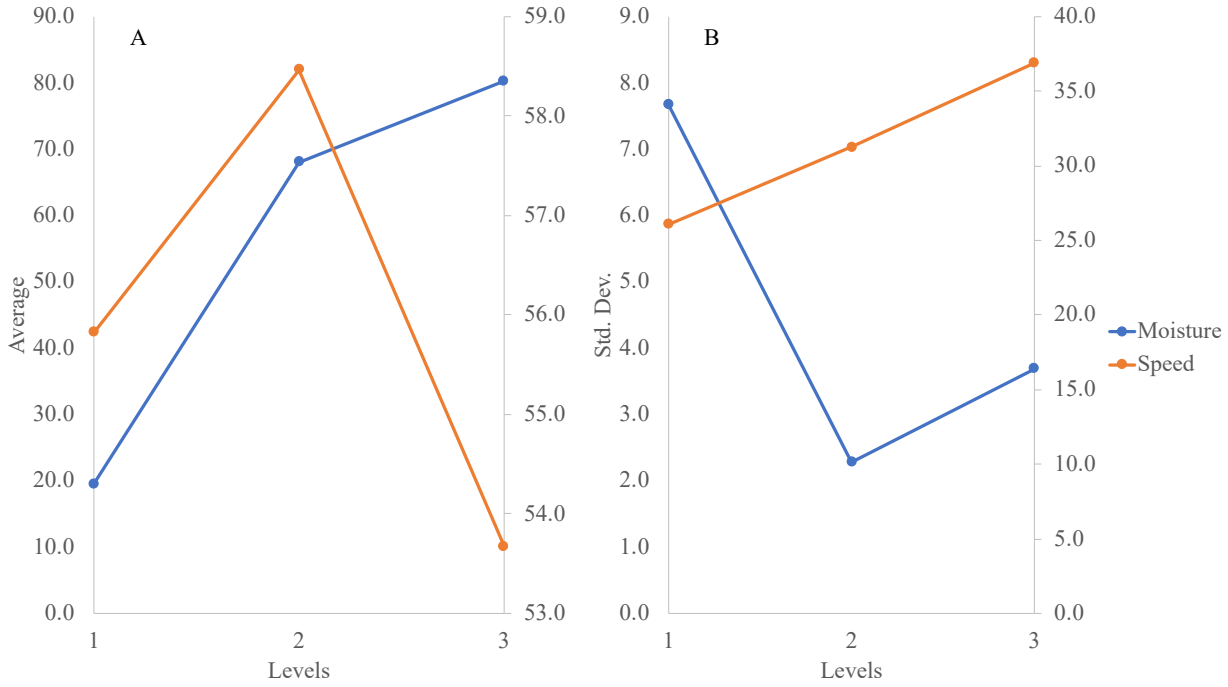

Figure 4.2: Individual effects of moisture content and motor frequency on average and standard deviation on percent aflatoxin degradation during extrusion.

Figure 4.4 displays total percent aflatoxin degradation as a function of the calculated residence time at each processing condition. As residence time increases, aflatoxin degradation seems to increase logarithmically towards 100% degradation. The relationship between aflatoxin degradation also helps to explain the relationship between aflatoxin degradation, feed rate, and temperature during processing. Figure 4.5 compares aflatoxin degradation with feed rate. As feed rate increases, aflatoxin degradation decreases. Higher feed rates decrease the residence time and thus, the time the product is exposed the shear forces that result in aflatoxin degradation.

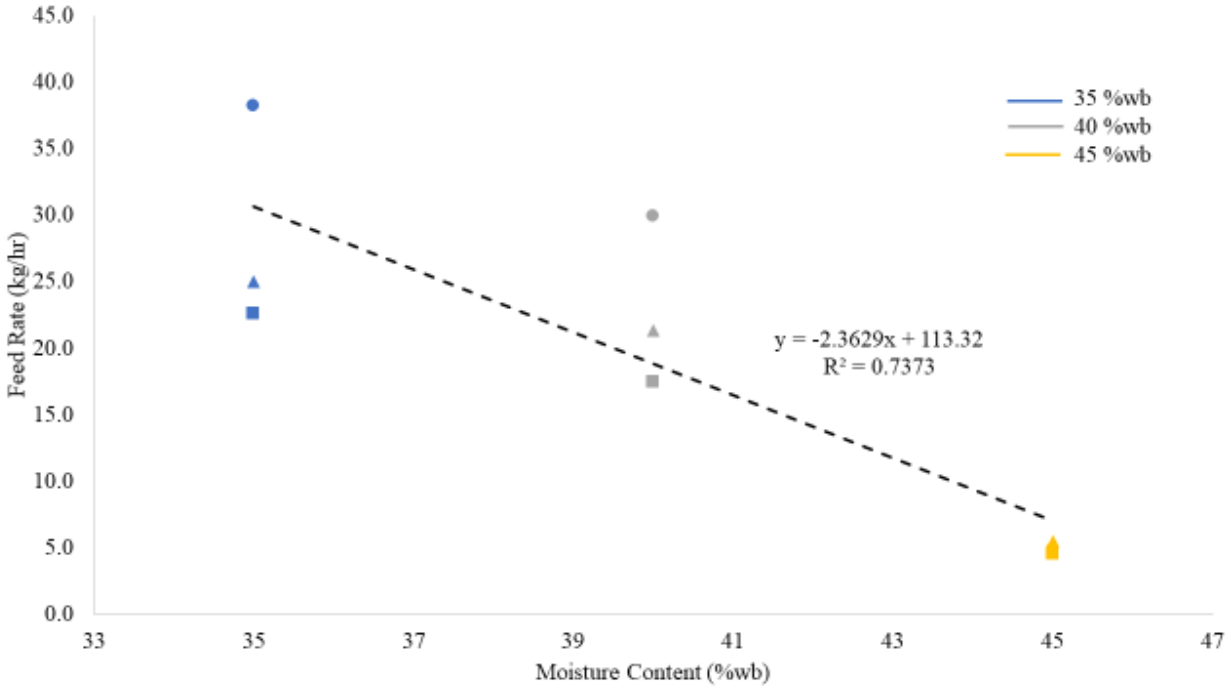

Figure 4.3: Feed rate (kg/hr) inside the extruder versus moisture content (%wb).

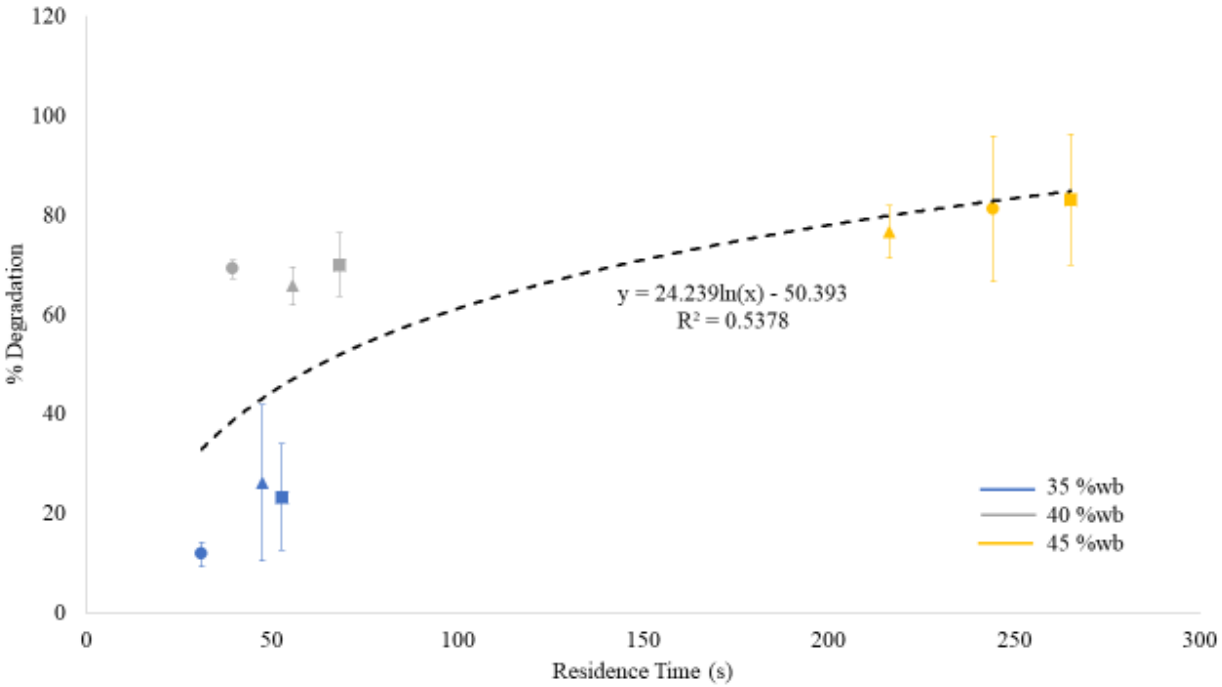

Figure 4.4: Total aflatoxin percent degradation versus residence time (s).

Further analysis on the role of residence time helps to explain the counterintuitive results when comparing maximum product temperature and aflatoxin degradation as seen in Figure 4.6. As seen in Figure 4.6, aflatoxin degradation at 35 %wb is comparably lower than degradation at higher moisture contents, despite having equal if not greater maximum temperature values. However, as previously mentioned, lower moisture content products experience decreased residence times. Thus, while products may experience higher temperatures as the result of greater shear forces due to increased viscosity, the products exposure to these conditions are limited. Thus, products at 35 %wb moisture achieve higher maximum temperatures yet less aflatoxin reduction.

Table 4.3: Approximate residence time (s) at each extruder operating condition.

Symbol	Motor Speed (hz)	Moisture (%wb)	Avg. Feed Rate (kg/hr)	Residence Time (s)
▲	15	35	25.0	47.6
■	38		22.6	52.6
●	50		38.2	31.1
▲	15	40	21.3	55.7
■	38		17.4	68.2
●	50		29.9	39.7
▲	15	45	5.5	216.4
■	38		4.5	265.1
●	50		4.9	244.4

It is also important to note that temperatures of at least 100 °C are needed for prolonged periods of time to degrade aflatoxin thermally. Thus, it can be assumed that negligible amounts of thermal degradation occur when maize at moisture contents between 35 and 45 %wb is processed using small-scale extrusion at varying motor frequencies (15 – 50 hz). This finding agrees with the results seen in Figure 4.6, where there are little to no degradation increases even with increased temperatures because the maximum temperature never goes above 100 °C. Additionally, from literature, degradation of aflatoxin becomes increasingly difficult at lower moisture contents during physical processing (Doyle, Applebaum, Brackett, & Marth, 1982). This is likely because moisture plays a critical role in hydrolyzing the lactone ring structure of aflatoxin, which is important for efficient degradation of the mycotoxin (Samarajeewa et al., 1990).

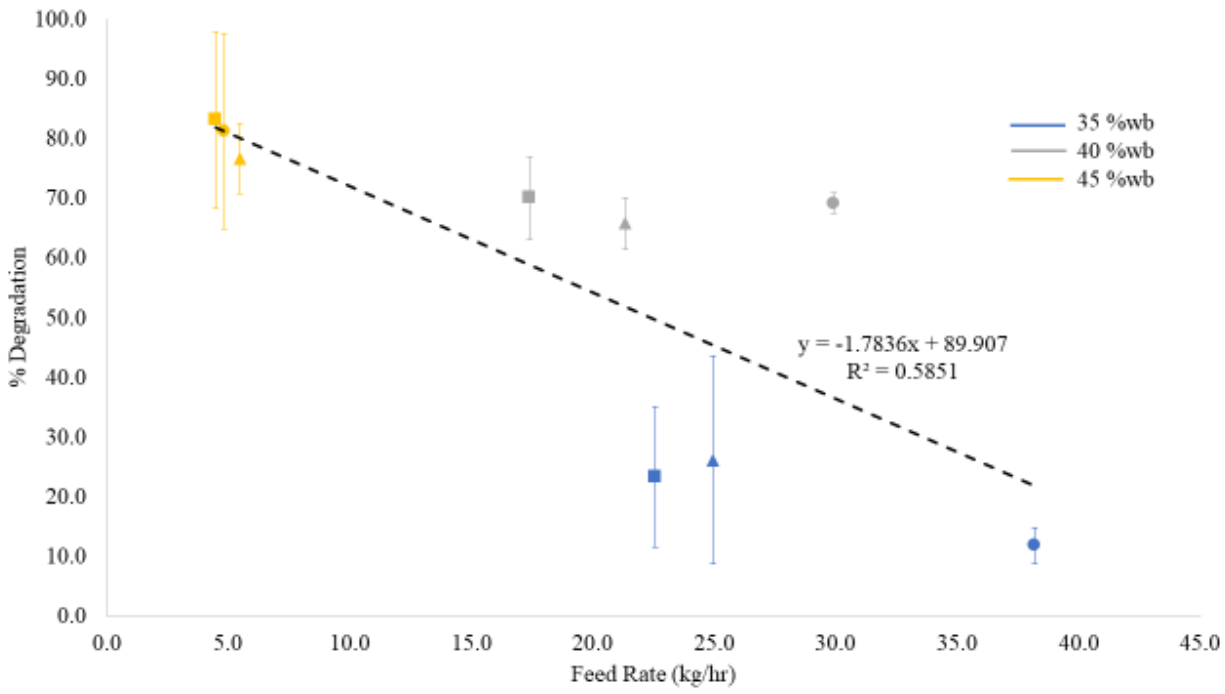

Figure 4.5: Total aflatoxin percent degradation versus feed rate (kg/hr).

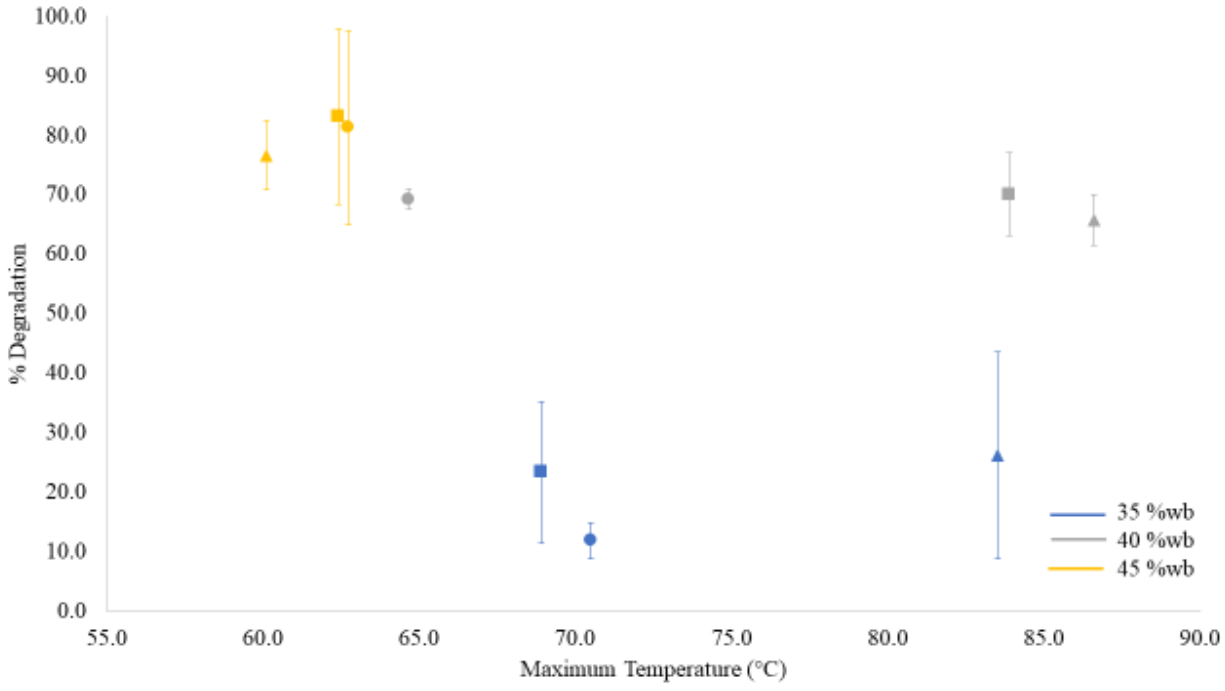

Figure 4.6: Total percent aflatoxin degradation versus maximum product temperature (°C).

4.6 Conclusion

Small-scale extrusion at varying motors frequencies (15 – 50 hz) and moisture content (35 – 45 %wb) can achieve total percent degradation between 11 and 83 % for aflatoxins in maize flour. Optimal aflatoxin degradation occurs at a moisture content of 43 %wb and motor frequency of 45 hz. Moisture content plays a significant role in total aflatoxin degradation (p-value < 0.05) as well as influences maximum temperature, feed rate, shear forces, viscosity, and residence time inside the extruder. Lower moisture contents (e.g. 35 %wb) contribute to increased viscosity and thus require increased feed rates to prevent from burning. As such, products at lower moisture contents experience shorter residence time inside the extruder, decreasing the time the product is exposed to thermal energy and shear forces. As a result, aflatoxin degradation decreases with increasing feed rates during extrusion processing. Thermal degradation under these conditions is likely negligible given that maximum product temperatures fail to reach the minimum temperature required to thermally degrade aflatoxin (100 °C). To optimize extrusion's effect on aflatoxin degradation, longer processing times should be utilized at higher moisture contents. However, more research is necessary to understand the role of differing shear rates at a constant moisture content and its relationship to aflatoxin degradation.

5. EFFECT OF EXTRUSION PROCESSING CONDITIONS ON AFLATOXIN DEGRADATION IN MAIZE PRODUCTS

5.1 Introduction

It is well known that temperature alone (e.g. roasting) is not a viable method for aflatoxin degradation in contaminated foods (Bullerman & Bianchini, 2007; Kabak, 2009b). While extrusion may expose a product to heightened temperatures depending on certain processing parameters, it also subjects food products to significant levels of shear that contribute to molecular reformation, chemical reactions, and the improvement of bioavailability in many products. In maize, extrusion is well known for its ability to gelatinize starch through changes to the protein structure (Tonner, 2018). Varying effects on protein, vitamins, and other molecules have also been recorded (Elias-Orozc et al., 2010; Gat & Ananthanarayan, 2015; Khaira, 2015; Pelembe, Erasmus, & Taylor, 2002; Sumathi et al., 2007). The effect of extrusion on aflatoxin degradation has been well-recorded, especially in products like peanuts (Gray, 2019; Molla & Zegeye, 2014; Onyango et al., 2005; F K Saalia & Phillips, 2011; Zheng et al., 2015). However, there is little literature quantifying the effect of shear on aflatoxin decontamination during extrusion, especially under high moisture and low temperature conditions. To better understand the role of extrusion on aflatoxin degradation, data from Chapter 4 was utilized to predict the contribution of temperature and shear to aflatoxin degradation in maize flour products in the Mini-Extruder™.

5.2 Predicting Temperature Effects on Aflatoxin Degradation

At very high temperatures, aflatoxin can undergo structural changes that render it less toxic upon consumption. However, even at high temperatures (>200 °C), aflatoxin requires a long exposure to be degraded to safe consumable levels (Bullerman & Bianchini, 2007). This is true of many products such as peanuts, pistachios, and some other tree nuts. In maize, limited studies have been conducting on the effect of temperature alone (e.g. roasting) on aflatoxin decontamination. This is likely because maize products are not often consumed in a roasted form and other more

advanced methods of thermal deactivation like microwave heating have been identified as more practical methods for decontamination. However, to fully understand effect of extrusion processing – which employs both high shear and temperature during processing - the contribution of temperature exposure to deactivation must first be determined.

5.2.1 Thermal Kinetics of Aflatoxin Degradation

There are very few studies on the decontamination of aflatoxin-infected maize products using temperature alone. Conway et al (1978) conducted experiments using maize grits and found that 40 – 80% of aflatoxin could be degraded at temperatures varying from 145 – 165 °C . Unfortunately, data regarding the length of roasting and the time-related samples is not available from the Conway et al study. However, studies on the kinetics of aflatoxin during roasting for peanut products are more prevalent. Martins et al (2017) studied the degradation of aflatoxin in peanuts while roasting at temperatures varying from 160 to 200 °C. At these temperatures, aflatoxin degradation ranged from approximately 61 to 87 % (Martins et al., 2017). Assuming that the kinetics of aflatoxin degradation does not change considerably between food products, data from Martins et al can be used to determine a relationship between thermal heating and aflatoxin degradation. This relationship can later be applied to predict the amount of aflatoxin degradation occurring inside the mini-extruder as a result of temperature alone.

Calculating the Thermal Rate Constant, k

For the purpose of this research, it is assumed that aflatoxin degrades from its toxic form to a less toxic form via a first order reaction. For a first order reaction, the integrated rate law can be used to determine the value of the rate constant, k, at varying temperatures. Equation 5 provides the integrated rate law for a first order reaction.

$$\ln\ [A] = -kt + \ln[A_o] \tag{5}$$

Where A is the the final concentration in moles, k is the rate constant in 1/s, t is time in seconds, and A_o is the initial concentration in moles. Since AFB1 aflatoxin is considered the most toxic and

a well-studied form of aflatoxin, its properties are used to estimate aflatoxin concentration (specifically molecular weight, Mw = 312.27 g/mol) and predict the thermal degradation of total aflatoxins using the Martins et al data.

From the Martins et al data, a graph of the natural log of the aflatoxin concentration versus time at varying temperatures (160, 180, and 200 °C) was created using Equation 5. The results of these calculations can be seen in Figure 5.1. From Equation 5, it is known that the negative slope of this line is equivalent the rate constant, k. Thus, the value of the rate constant k at 160, 180, and 200 °C is equivalent to 0.0006, 0.0009, and 0.0012 1/s respectively. The value of the rate constant increases with temperature, which follows chemical reaction kinetic theory starting that more energy applied (e.g. higher temperatures) help a reaction proceed at a faster rate.

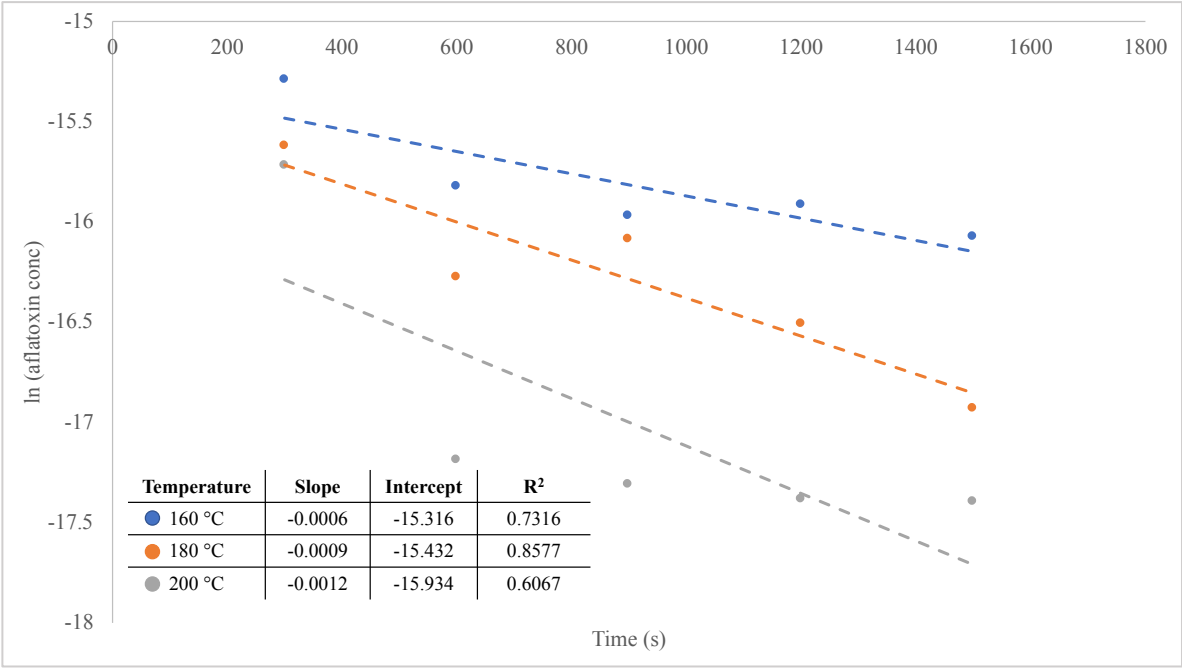

Figure 5.1: The natural log of aflatoxin concentration versus time (s).

Determining Activation Energy, E_A

The values of the rate constants, k, determined in the previous section can be used to calculate the activation energy, E_A, for the degradation of aflatoxin. The activation energy can be

calculated by graphing the natural log of the reaction rate versus temperature as seen in Figure 5.2. Figure 5.2 uses a manipulated version of the Arrhenius Equation as seen in Equations 6 and 7.

$$k = Ae^{-\frac{E_A}{RT}} \tag{6}$$

Where A is the pre-exponential factor, E_A is the activation energy in joules, R is the universal gas constant in J/mol/K, T is temperature in Kelvin, and k is the rate constant in 1/s. Equation 2 can be linearized to create Equation 3 by taking the natural log of both sides of the Arrhenius Equation.

$$\ln k = \ln A - \frac{E_A}{RT} \tag{7}$$

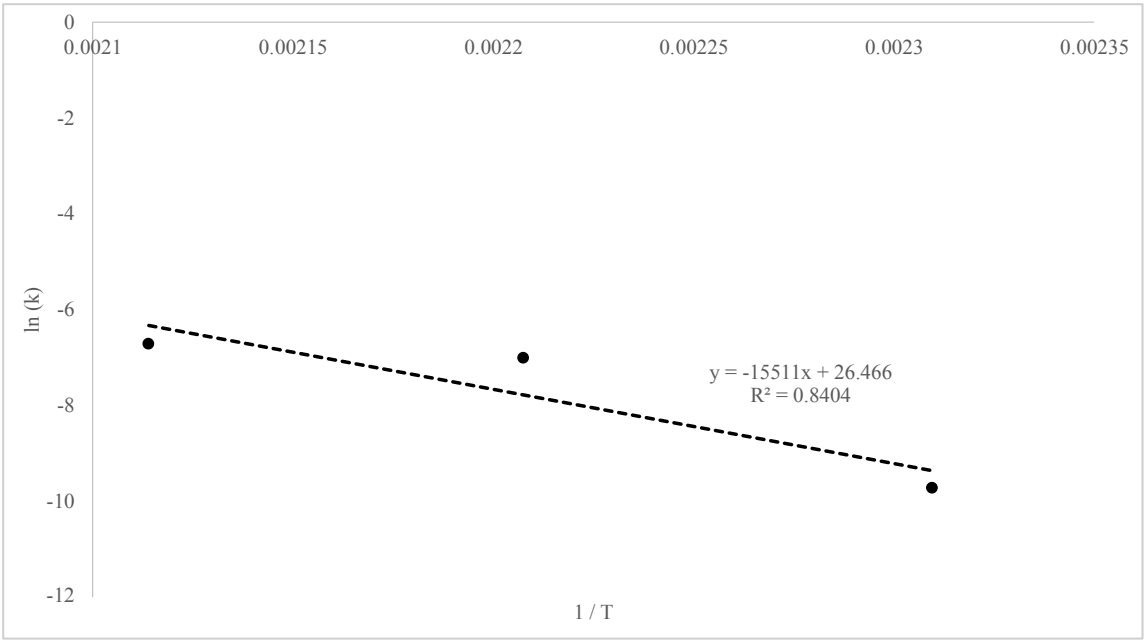

Figure 5.2: Natural log of the rate constant, ln(k), versus inverse temperature (1/T)

When graphing ln(k) versus the inverse temperature, the slope of the line created using Equation 3 is equivalent to $-E_A/R$. Multiplying the slope by R and taking the absolute value, one can solve for the activation energy, E_A. From Figure 5.2 the slope is determined as 11551 K. Multiplying this value by 8.314 J/mol/K, the activation energy for aflatoxin degradation is approximately $1.29 * 10^5$ J/mol. This value aligns well with data for the activation energy determined for rice, corn, and peanuts (Zhang et al, 2011). Given that this activation energy falls

within the same order of magnitude of other research studies, it is assumed that it is an appropriate value to approximate the effect of temperature on aflatoxin in maize. Equation 8 represents the equation relating rate constant and temperature, which can later be used to determine the rate constant values along the length of the extruder and ultimately, thermal aflatoxin degradation.

$$\ln k = -\frac{11551}{T} + 26.466 \tag{8}$$

5.2.2 Calculating Aflatoxin Degradation due to Temperature inside the Mini-Extruder™

Estimating Temperature Profiles inside the Extruder

The temperature inside an extruder is not isothermal. Instead, temperature often increases along the length of the extruder barrel. It can be difficult to accurately determine the true temperature profile inside an extruder, since product often becomes stuck to any thermocouples used and this can distort the temperature measurements (Penner, 2011). To resolve this issue, it is convenient to assume a temperature profile beginning at ambient temperature (~23.2 °C) and increasing linearly to the die temperature, which can be more easily measured using thermocouples. For the purpose of this work, a linear temperature profile was assumed. However, while this approximation is suitable for this work, it is important to note that product temperatures inside the extruder barrel could exceed those temperatures measured at the die.

The temperature profile inside the extruder varies with screw speed and moisture content of the material passing through. Thus, a unique temperature profile must be created for each operating condition where maize flour was tested for aflatoxin degradation. Figure 5.3 shows the temperature profile along the length of the extruder (0.30 m) for each operating condition. The temperature profile was solved for using length increments of 0.01m. In general, temperature increases are greater for conditions with lower moisture content. This is likely a result of high moisture content creating lower viscosity and thus, less shear stress increasing temperature inside the extruder.

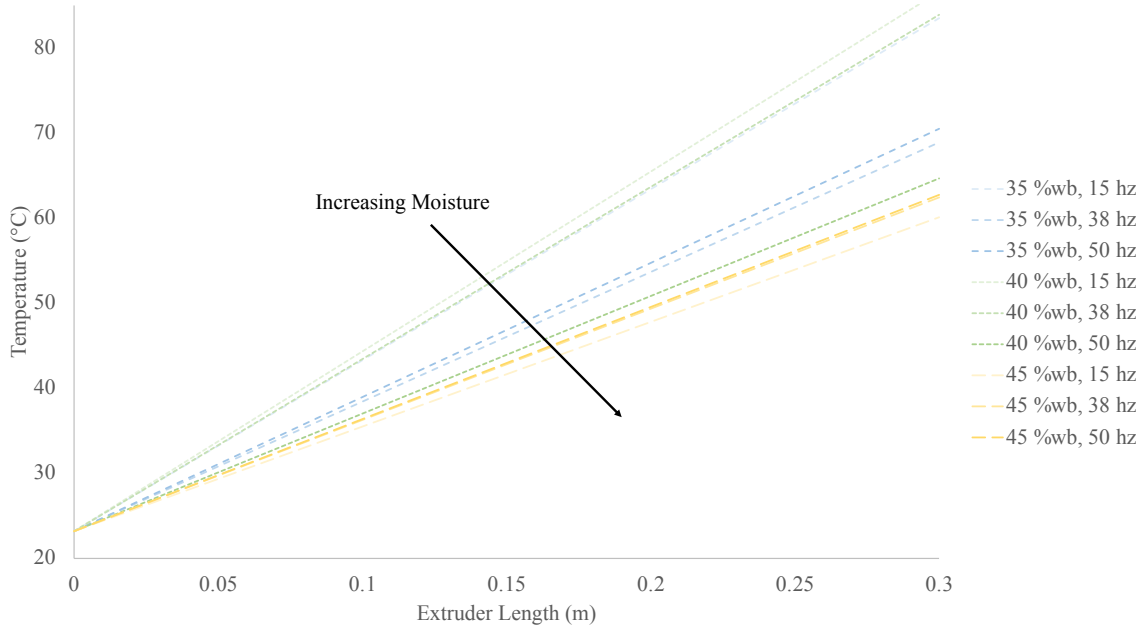

Figure 5.3: Temperature profiles inside the extruder at different operating conditions.

Determining Thermal Degradation of Aflatoxin in the Extruder

The temperature profiles from the previous section and Equation 8 can be used to solve for the rate constant along the extruder at each operating condition. Using these values of the rate constant in addition to the activation energy (see Section 5.1), time, and initial concentration of aflatoxin estimates for thermal degradation of aflatoxin can be determined. Time was determined by dividing the average residence time under each operating condition as calculated in Chapter 4 by the length of the extruder (m) and then multiplying it by the length at a certain point in the extruder. The process was repeated for each set of processing condition (i.e. runs 1 – 9) where moisture content and screw speed are varied. The percent of aflatoxin degradation due to temperature was then calculated using the initial and final aflatoxin concentrations in molar concentration (M).

Figure 5.4 shows the relationship between the maximum temperature for each run (e.g. the die temperature) and the thermal percent degradation of aflatoxin. There is a clear increase in degradation as temperature increases. This directly contrasts with the overall trend seen in Figure 4.2 from the previous chapter, where an increase in temperature correlated with a decrease in total aflatoxin percent degradation. This discrepancy indicates that another variable or variables must

be influencing the degradation of aflatoxin beyond temperature. Given that extrusion also applies a significant amount of shear to a given product, it is assumed that the shear forces supply the remainder of the energy utilized to degrade aflatoxin inside the extruder. Since the ability of shear to degrade aflatoxin inside the extruder is highly dependent on feed rate and residence time, it follows that – while higher temperatures may be more effective for thermal degradation – they simultaneously correlate with higher feed rates and thus, a lower residence time. The dominance of the shear effect is further reiterated by the fact that at temperatures less than ~ 80°C, the percent change in aflatoxin remains less than one thousandth of a percent. The complete percent degradation due to temperature along the extruder length can be found in Appendix E.

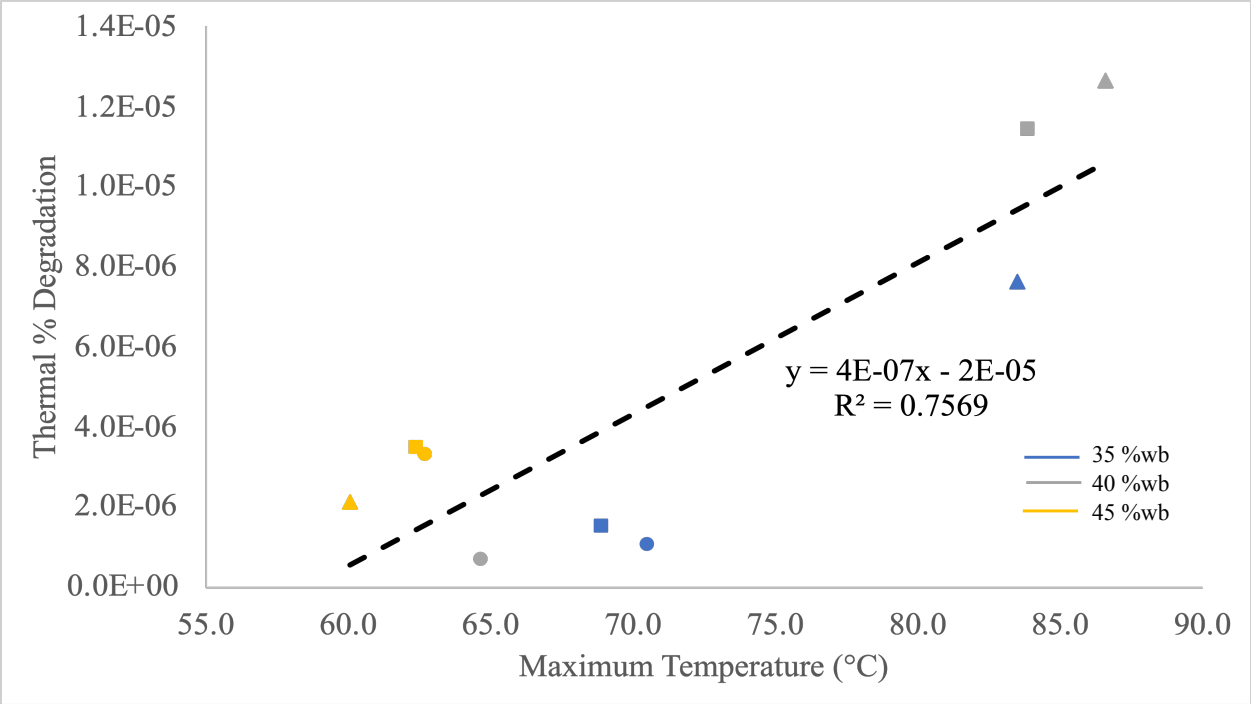

Figure 5.4: Thermal percent aflatoxin degradation versus maximum die temperature (°C).

5.3 Predicting Effects of Shear on Aflatoxin Degradation

During extrusion cooking, products can reach very high temperatures. High temperatures are achieved by pushing the food product – often a dough-like mixture – through a small channel or barrel via a rotating screw. As a result, high temperatures (>150°C) and shear forces can be generated by the extrusion process (Bullerman & Bianchini, 2007). However, in small-scale

extruders and with high moisture products, such high temperatures are difficult to achieve. As described in Section 5.2, this renders the effects of temperature obsolete inside the mini-Extruder™. However, a reduction in total aflatoxins was still seen post-extrusion processing. This is likely the result of shear forces applied to the product, which are known to contribute to a various number of molecular modifications to compounds inside extrudates (Bullerman & Bianchini, 2007). As such, understanding the contribution of shear forces to aflatoxin degradation inside the extruder is critical for improving future extruder designs and optimizing aflatoxin reduction.

5.3.1 Determining Viscosity Inside the Extruder

The study of rheology is dedicated to understanding viscosity, which is best described as a fluid's resistance to flow. Inside the extruder, viscosity of a given product is a function of shear rate, moisture content, and temperature (Harper, 1981). Viscosity and shear rate can be used to calculate shear stress on a given product. As such, determining viscosity at each operating condition, where shear rate and moisture content are varied, is critical to relating the amount of shear stress inside the Mini-Extruder and its relationship to aflatoxin degradation.

To calculate viscosity at each operating condition, a master curve for yellow corn at range of temperatures (80 - 120 °C), shear rates, and moisture contents (35 – 40 %wb) was used (Ponrajan, 2016). Given the complexity of extruder geometry and the limited data on small-scale extrusion, it was assumed that the extrapolation of Ponrajan's master curve was sufficient for gaining a good understanding of the behavior of maize flour inside the Mini-Extruder for this research. Equation 9 represents the final equation utilized by Ponrajan's master curve.

$$\ln \eta = m[\ln \dot{\gamma} + \ln a1 + \ln a2 + \cdots + \ln ai] + b \tag{9}$$

Where η is viscosity in Pa*s, m is the slope, λ is the shear rate in 1/s, a1, a2, ….. , ai are the shift factors determined using empirical data, and c is the intercept. For Ponrajan's master curve, there are only two shift factors, aM and aT, which represent the shift factor due to moisture content (%wb) and temperature (°C) respectively. Substituting in aM and aT, Equation 5 simplifies to become Equation 10.

$$\ln \eta = m[\ln \dot{\gamma} + \ln aM + \ln aT] + c \quad (10)$$

The shift factors aM and aT are calculated using Equations 11 and 12 respectively.

$$\ln aM = 19.638\,(M + b) - 6.8518 \quad (11)$$

$$\ln aT = 0.0355T - 2.8868 \quad (12)$$

Where M is the product moisture content in %wb, T is temperature in °C, and b is calculated using Equation 13.

$$b = -0.0002T + 0.0153 \quad (13)$$

Note that for the purposes of this research, an average temperature was used to determine viscosity of the product inside the extruder at each operating condition. The maximum temperature different in the product was ~60 °C. It is assumed that the average viscosity changes minimally with such small temperature changes. Assuming that the moisture content of the product is constant and that the temperature change of the product creates a negligible change in viscosity within the extruder, the Equation 14 can be used to determine the average temperature.

$$T_{avg} = \frac{(T_1 + T_2)}{2} \quad (14)$$

Where T_1 is the ambient temperature of the product before processing and equal to 23.2 °C, T_2 is the maximum temperature in °C of the product exiting the extruder. Finally, substituting in the value of constants m and c from Ponrajan's work, Equation 10 becomes Equation 15.

$$\ln \eta = -0.7441[\ln \lambda + \ln aM + \ln aT] + 11.147 \quad (15)$$

It is important to note that the version of Equation 15 used from Ponrajan's work is the No Bagley version, meaning that the Bagley corrected version of the master curve is not utilized.

Ponrajan found that the applying the Bagley correction did not significantly impact the viscosity master curve for whole yellow corn under the range of conditions test. R^2 values when moving removing the Bagley correction drop only from 0.99 to 0.97, indicating that the Bagley correction can be omitted (Ponrajan, 2016).

As seen in Equation 11, viscosity is also dependent on shear rate inside the extruder. To determine shear rate at various points in the extruder, Equations 16 through 19 were utilized (Harper, 1981). Equation 16, 17, 18, and 19 represent the shear rate inside the extruder channel, clearance, die, and steam lock respectively.

$$Channel\ \dot{\gamma} = \frac{\pi \cdot D \cdot N}{60 \cdot H} \tag{16}$$

$$Clearance\ \dot{\gamma}_{\delta} = \frac{\pi \cdot D \cdot N}{60 \cdot \delta} \tag{17}$$

$$Die\ \ \dot{\gamma}_{die} = \ \frac{3n+1}{4n} \cdot \frac{4 \cdot F}{\pi \cdot \rho \cdot R^3} \tag{18}$$

$$Steam\ Lock\ \ \dot{\gamma}_{sl} = \frac{\pi N sl}{sl_{gap}} \tag{19}$$

Where D is the internal barrel diameter (accounting for the clearance) in mm, N is the screw rotational speed in rpm, H is the channel depth in mm, δ is the clearance between the barrel and the screw in mm, n is the flow behavior index, ρ is the product density in kg/m^3 calculated using the Choi-Okos equation, R is the radius of the medium die in mm, and F is the product feed rate in kg/s (Harper, 1981; Onita & Ivan, 2005; Rauwendaal, 1994). Specific values for each extruder dimension can be found in Appendix F.

The percentage of material passing through the screw and the clearance of the extruder at steady state are 89.7 and 10.3% respectively (Penner, 2011). Using these percentages, a weighted average shear rate for each operating condition can be calculated as seen in Table 5.1. Note that motor speed (rpm) was converted to screw speed (rpm) by using a reduction ratio of the largest screw speed over the largest motor speed. Using data from Tonner, the maximum screw speed was determined to be ~100 rpm at temperatures between 60 and 80 °C. The maximum motor speed is 3000 rpm. Thus the reduction ratio used is 100/3000 = 0.033. It is also important to note that

Equations 16 and 17 provide only rough estimates for shear rate and assume Newtonian behavior of the fluid inside the extruder. However, this estimate of the shear rate can be used to determine the amount of energy being applied to the material as seen in Section 5.3.2 and later, is used to determine an effective shear rate.

Table 5.1: Viscosity calculations at various extruder moisture content (35, 45, 45 %wb) and motor speed (15, 38, 50 hz).

	Operating Conditions					
Symbol	**Motor Speed (Hz)**	**Moisture Content (% wb)**	**Screw Speed, N (rpm)**	**Average Temp, T (°C)**	**Newtonian Shear Rate, $\dot{\gamma}$ (1/s)**	**Newtonian Viscosity, η (Pa*s)**
▲	15	35	30.0	53.4	16.5	1.8E+04
■	38		76.0	46.1	41.7	1.1E+04
●	50		100.0	46.9	54.9	8.5E+03
▲	15	40	30.0	54.9	16.5	8.4E+03
■	38		76.0	53.6	41.7	4.3E+03
●	50		100.0	43.9	54.9	4.4E+03
▲	15	45	30.0	41.7	16.5	5.6E+03
■	38		76.0	42.8	41.7	2.7E+03
●	50		100.0	43.0	54.9	2.2E+03

5.3.2 Determining Effective Shear Rate from SEC

It is assumed that the viscosities calculated in Section 5.3.1 can be utilized to determine a rough estimate of the Specific Energy Consumption (SEC) at each operating condition. As seen in Equation 20, the total energy inside the extruder is a function of the energy due to viscous dissipation in the channel, energy to raise the pressure in the fluid, viscous energy dissipation in the flight clearance, viscous energy dissipation in the steam locks, energy dissipation due to back mixing from the pressure build-up from the die, and energy dissipation within the die (Harper, 1981; Penner, 2011).

$$E(\dot{\gamma}) = p \cdot \frac{(\pi DN)^2 L}{sin\theta}\left[\eta \cdot \frac{W}{H}(\cos\theta^2 + 4\sin\theta^2) + \eta_\delta \cdot \frac{e}{\delta} + \cdot \eta_{sl} \cdot \frac{sl}{sl_{gap}}\right] + p \cdot \frac{\pi DNWH}{2} \cdot \Delta P_{backmix} \cos\theta + \dot{\gamma}_{die} \cdot \eta_d \cdot Q \quad (20)$$

Where,

$$\Delta P_{backmix} = \frac{G1 \cdot Fdt}{\frac{K}{\eta_d} + \frac{G2 \cdot Fdt}{\eta \cdot L}} \tag{21}$$

And the values of G1, G2, and K can be calculated using Equations 22 through 24.

$$G1 = \pi^2 D^2 H \left(1 - \frac{e \cdot p}{\pi \cdot D \cdot \sin\theta}\right) \sin\theta \cos\theta \tag{22}$$

$$G2 = \frac{\pi}{12} \cdot D \cdot H^3 \left(1 - \frac{e \cdot p}{\pi \cdot D \cdot \sin\theta}\right) \sin\theta^2 \tag{23}$$

$$K = \frac{\pi\left(\frac{D_{die}}{2}\right)^2}{8 \cdot L_{die}} \tag{24}$$

Note that $E(\dot{\gamma})$ in Equation 20 are J/s (Watts). Equation 16 is Harper's energy equation describing the total energy dissipation, which is determined by integrating each component over the length of the channel. Appendix F provides a description for each variable and its associated value as well as Harper's equation for total energy dissipation inside the extruder.

The total energy dissipation described in Equation 16 can be equated to the Specific Energy Consumption (SEC). SEC is calculated and assumed to be equal to Specific Mechanical Energy, as seen in Equation 25 (Penner, 2011).

$$SEC = \frac{t_{res} \cdot \dot{\gamma}_{eff}^2 \cdot \eta(\dot{\gamma})}{\rho} \tag{25}$$

Where t_{res} is the average residence time in s for each operating condition as calculated in Chapter 4, $\dot{\gamma}_{eff}$ is the effective shear rate accounting for the non-newtonian behavior of the product in 1/s, η is the viscosity in Pas from Table 2 calculated in Section 5.3.1 in Pas, and ρ is the product density in kg/m^3 calculated using the Choi-Okos Equation.

Next, it is assumed that the amount of total energy calculated in Equation 16 divided by the volumetric flow rate, F, in kg/hr and product density can be equated to Equation 21. This creates Equation 26, where the effective shear rate ($\dot{\gamma}_{eff}$) is the only unknown.

$$\frac{t_{res}\cdot\dot{\gamma}_{eff}^{2}\eta(}{\rho} = \frac{E(\dot{\gamma})}{F*1000} \tag{26}$$

Where F is the mass flow rate of the product inside the extruder in kg/hr. Using Excel's Solver tool, the left and right hand side of Equation 22 can be equated for each operating condition and used to solve for the value of $\dot{\gamma}_{eff}$. Using the value of $\dot{\gamma}_{eff}$, an effective viscosity is then calculated using the same method described in Section 5.3.1 (Ponrajan, 2016). Table 5.2 provides the values the new values for shear rate and viscosity at each operating condition.

Included also in Table 5.2 is the energy entering the system in the form of sensible heat (e.g. thermal energy). In theory, the SEC should be equivalent to the amount of thermal energy exchanged to the product via the temperature change. Sensible heat, Q in J/hr, is calculated in Equation 27.

$$Q = \dot{m}C_p\Delta T \tag{27}$$

Where $\dot{m}$ is equivalent feed rate, F, in kg/hr, C_p is heat capacity (J/kg/K) and ΔT is the temperature change in °C/Kelvin from the ambient temperature (23.2°C) to the maximum die temperature at each operating condition . This value can be compared to the value of SEC calculated in Equation 25 by dividing Q by the feed rate, F, and converting J/hr to kW to achieve units of kW*hr/kg.

Table 5.2: Effective viscosity and shear rate inside the extruder as a function of SME/SEC

	Operating Conditions					
Symbol	**Motor Speed (Hz)**	**Moisture Content (% wb)**	**SEC (kW*hr/kg)**	**Sensible Heat (kW*hr/kg)**	**Effective Shear Rate, $\dot{\gamma}$ (1/s)**	**Effective Viscosity, η (Pa*s)**
▲	15	35	0.32	0.15	43.6	8.6E+03
■	38		0.38	0.11	59.2	8.2E+03
●	50		0.41	0.12	89.4	5.9E+03
▲	15	40	0.35	0.15	58.4	3.3E+03
■	38		0.39	0.15	77.7	2.7E+03
●	50		0.42	0.10	106.2	2.7E+03
▲	15	45	0.31	0.08	33.5	3.3E+03
■	38		0.53	0.09	56.5	2.2E+03
●	50		0.63	0.09	71.1	1.8E+03

5.3.3 Determining Shear Stress

Shear stress can calculated using the viscosity and the shear rate inside the extruder. Equation 28 calculates the average shear stress (τ) in Pa.

$$\tau = \eta(\dot{\gamma}) * \dot{\gamma} \tag{28}$$

Where η is viscosity in Pa.s is a function of shear rate as seen in Section 5.3.1 and $\dot{\gamma}$ is shear rate in 1/s. Table 5.3 provides the effective shear rate inside the extruder at each operating condition using the values for effective shear rate and the effective viscosity calculated in Section 5.3.2. The Newtonian shear stress, calculated from the Newtonian viscosity and shear rate is also included.

Table 5.3: Newtonian and effective Shear stress inside the extruder at each operating condition.

	Operating Conditions			
Symbol	**Motor Speed (Hz)**	**Moisture Content (% wb)**	**Newtonian Shear Stress (kPa)**	**Effective Shear Stress (kPa)**
▲	15	35	293.7	376.7
■	38		442.4	483.9
●	50		465.7	527.6
▲	15	40	137.7	190.4
■	38		180.4	211.5
●	50		242.6	287.3
▲	15	45	91.5	109.8
■	38		113.0	122.1
●	50		120.7	129.0

5.3.4 Modeling the Effects of Shear Forces on Aflatoxin Degradation

Zhao *et al* provide a series of models necessary to determine the additional effect of shear on the thermokinetic degradation of molecules like starch or thiamine in maize (Zhao et al., 2011). These models describe the change in chemical compounds as a function of shear and thermal kinetics inside the extruder. These models can be readily applied to the degradation of aflatoxin inside the Mini-Extruder©. However, in Section 5.2 it was determined that temperatures inside the extruder under these operating conditions has little effect on the aflatoxin degradation. The small effect of temperature is a result of minimal temperature increases during extrusion because of high product moisture contents. Thus, since thermal degradation is minimal, it is assumed that all aflatoxin degradation can be attributed to the shear forces endured during extrusion.

Equation 29 from Basedow and others details the relationship between the reaction constant for shear, k_s, and shear energy, E_s.

$$k_s = k_{s0}\exp\left(-\frac{E_s}{\tau V}\right) \tag{28}$$

Where V is an empirical material-related parameters without direct correspondence to real space and τ is the shear stress in Pa (Zhao et al., 2011). Zhao et al reiterates that since the parameter V is unknown, only relative values of activation energies (E_s/V) can be determined.

Equation 28 exactly models the Arrhenius Equation (see Equation 6), but replaces the parameters RT with values τV. It is assumed that aflatoxin degradation due to shear forces behaves as a first order reaction as seen in Equation 29. the integrated rate law form as seen in Equation 29 is developed.

$$Rate = \frac{d[C]}{dt} = -k_s[C] \tag{29}$$

Where C is the concentration of aflatoxin M, k is the rate constant in 1/s, and t is time in seconds. Equation 29 is known as the Basedow Model and is appropriate when the effects of temperature are considered negligible as determined in Section 5.1. Equation 29 can be rearranged into Equation 30.

$$\ln\left(\frac{[C_t]}{[C_0]}\right) = -k_S t \tag{30}$$

Where C_t is the final concentration of aflatoxin at time t in seconds and C_0 is the initial concentration of aflatoxin at time t = 0. Since the values of initial and final aflatoxin are known as well as the residence time, the rate constant k can be determined for each unique set of operating conditions. Next, similar to Section 5.2.1 where the Activation Energy (E_A) was determined by graphing ln (k) versus 1/T, the Shear Activation Energy ratio (E_s/V) can be determined by graphing the ln (k_s) versus 1/ τ as seen in Figure 5.5.

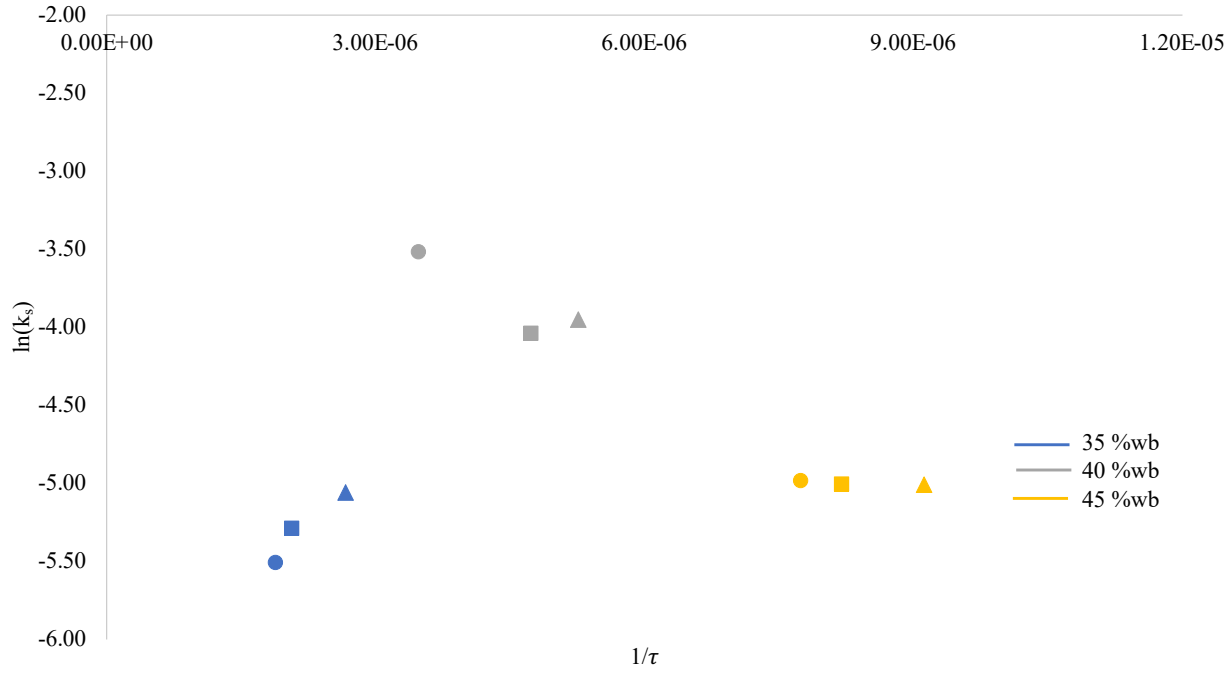

Figure 5.5: Natural log of the shear stress rate constant (k_s) versus inverse shear stress ($1/\tau$)

Similar to Section 5.1, where the amount of degradation due to temperature is calculated, the linear equation relating k_s and $1/\tau$ provides a relationship that allows for the determination of the kinetics of shear degradation of aflatoxin inside the mini-Extruder™ (Zhao et al., 2011). Unfortunately, as seen in Figure 5.5, the value of E_s/V is not easily deduced from a comparison of k_s and $1/\tau$ and the Basedow model is no longer applicable. Rather than a negative sloping line, the results in Figure 5.5 appear to have a maximum rate constant occurring at a moisture content around 40 %wb. This indicates that beyond shear stress, another factor – likely moisture content – is having a significant influence on the aflatoxin degradation. The implications of this finding are further discussed in the following section.

5.4 Results and Discussion

Understanding the effect of temperature and shear on aflatoxin degradation during extrusion processing is critical for improving small-scale extrusion food processing technology. As noted in Section 5.2, the small change between ambient and the maximum product temperature resulted in very little thermal degradation of aflatoxin as seen in Figure 5.4. However, it is

important to note that thermal degradation is also closely related to the product moisture and residence time inside the extruder. For this research, it was difficult to run any samples below 35 %wb moisture because products below this moisture content tend to burn inside the extruder. In contrast, high moisture contents (>40%) prevent any significant amount of shear from being applied to the product and the corresponding temperature change is very low. To reach the higher temperatures required for thermal aflatoxin degradation, optimizing the product moisture and shear rate (including geometry) will be of critical importance.

Since thermal degradation inside the Mini-Extruder™ was considered negligible, it is assumed that all degradation can be attributed to either a combination of thermal-shear interactions or to shear alone. Again, given the low temperature increase during processing, it is further assumed that shear forces alone are responsible for the degradation seen in aflatoxin levels pre and post-extrusion processing. Thus, identifying the relationship between aflatoxin degradation with specific mechanical energy (SME), shear rate, viscosity, and shear stress is of critical importance.

The results of SEC using the Harper Equation are graphed versus the effective shear rate as seen in Figure 5.6. This relationship is fitted using a power law equation and align well with results from Penner. As shear rate increases, the amount of energy increases via a power law relationship (Penner, 2011). The results in Figure 5.6 affirms that at higher shear rates, greater amounts of energy are applied to the product.

Figure 5.67 compares the SEC with the aflatoxin degradation at varying moisture contents. At higher moisture contents (e.g. 45 %wb) the product experiences higher levels of degradation as compared to lower moisture contents (e.g. 35 %wb). It is interesting to note that for 35 and 40 %wb moisture content, the sample degradation does not increase – and even drops in the case of products with 40 %wb moisture – as shear rate increases. However, the residence time for samples at high shear rates is shorter and therefore, the product is subjected to less time in the extruder and ultimately less shear. In contrast, samples at 45 %wb experience a higher residence time because their high moisture content prevented the product from moving quickly through the extruder even at high shear rates. Thus, product tested at 45 %wb spent on average the most time in the extruder and were thus subjected to conditions inside the extruder (e.g. shear rate) for a longer period of time. This indicates that, rather than shear rate itself, the amount of time the product spends in the extruder and the moisture of the product is critical, reiterating the results found in Chapter 4 where moisture content – not motor/screw speed – was determined to be significant.

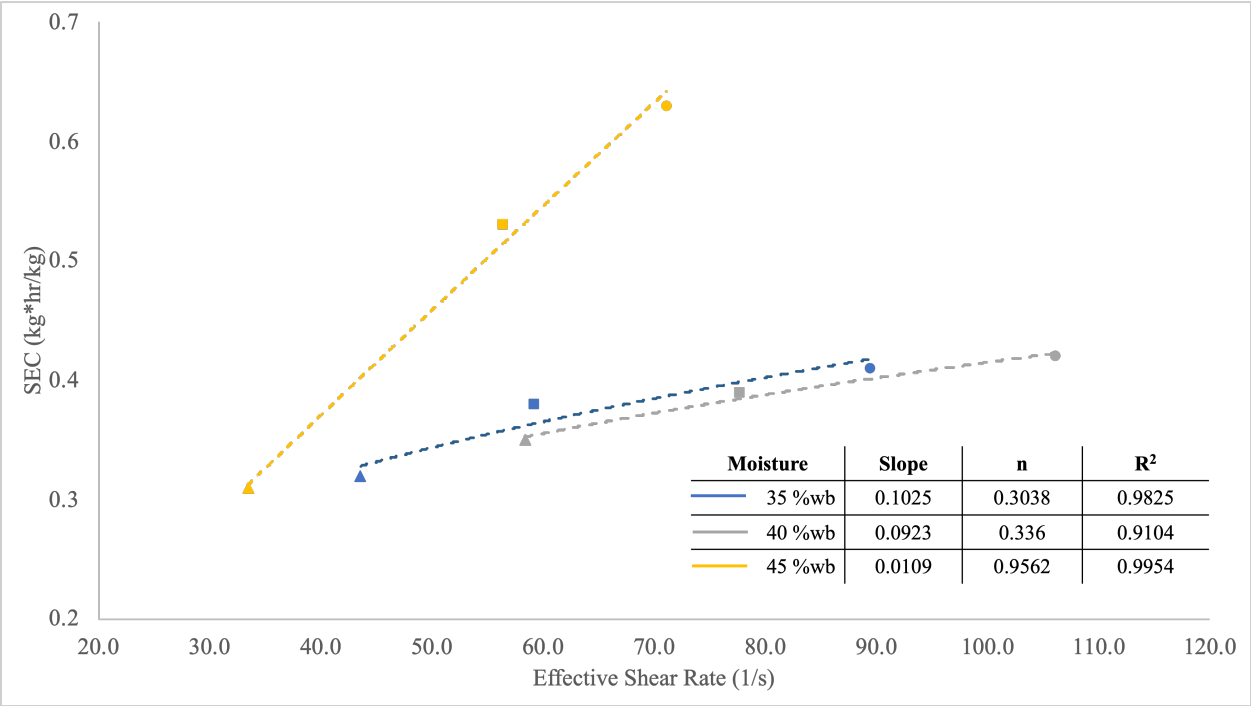

Figure 5.6: SEC as a function of shear rate at moisture content 35, 40, and 45 %wb.

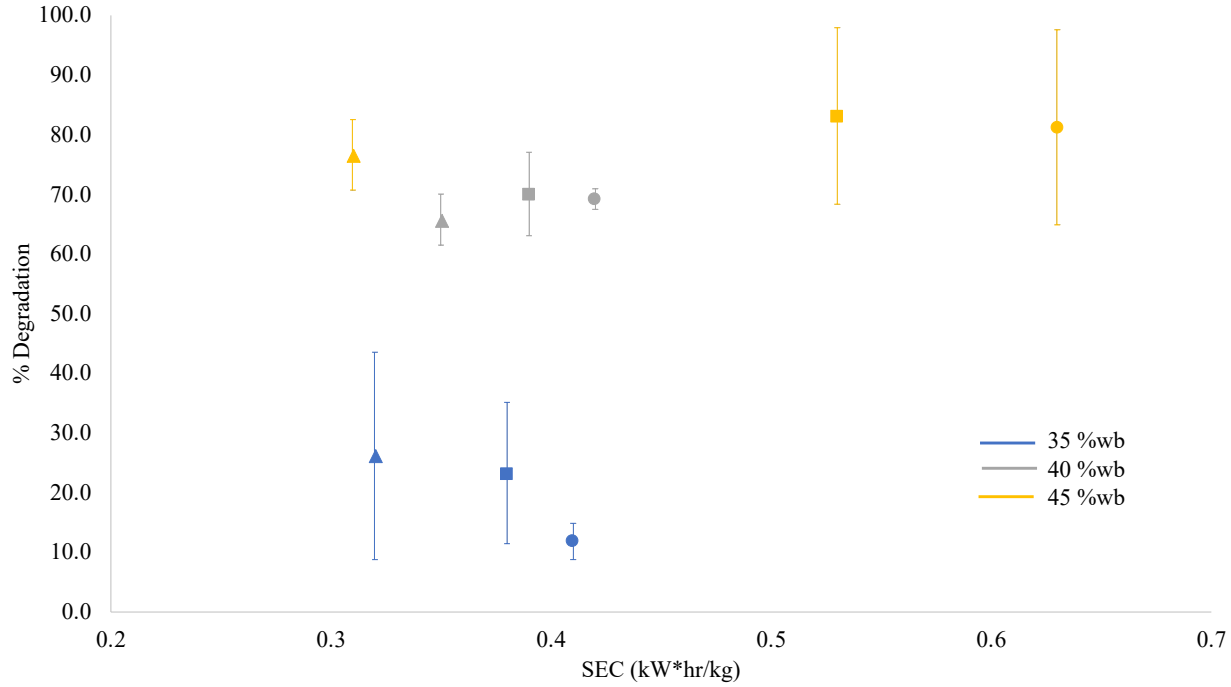

Figure 5.7: Total aflatoxin percent degradation versus Specific Energy Consumption (SEC).

It is important to note that the values for SEC and the sensible heat energy differ slightly when they are expected to be the same. While in the same order of magnitude (10^{-1}), SEC values calculated using Harper's Equation are slightly greater than those determined using the Sensible Heath Equation. There are a few possible reasons these values are not equivalent. First, the maximum temperature recorded at the die is not the actual temperature the product reaches inside the extruder. For this study, a linear temperature profile was assumed. However, given the geometry of extruders and increasing bushing size, the maximum temperature may be reached before the die. Second, it is possible that the recording of the thermocouple was not representative of the actual greatest temperature at the die due to product build up on the thermocouple. Penner recorded similar results when attempting to record a temperature profile of a small-scale extruder (60 pph) using thermocouples along the length of the extruder barrel (Penner, 2011). However, despite being of slightly different value, the SEC from the Harper Equation and the Sensible Heat Energy have similar trends as seen in Figure 5.8. Higher degradation percentages continue to occur at higher moisture contents rather despite the amount of energy gained by the system.

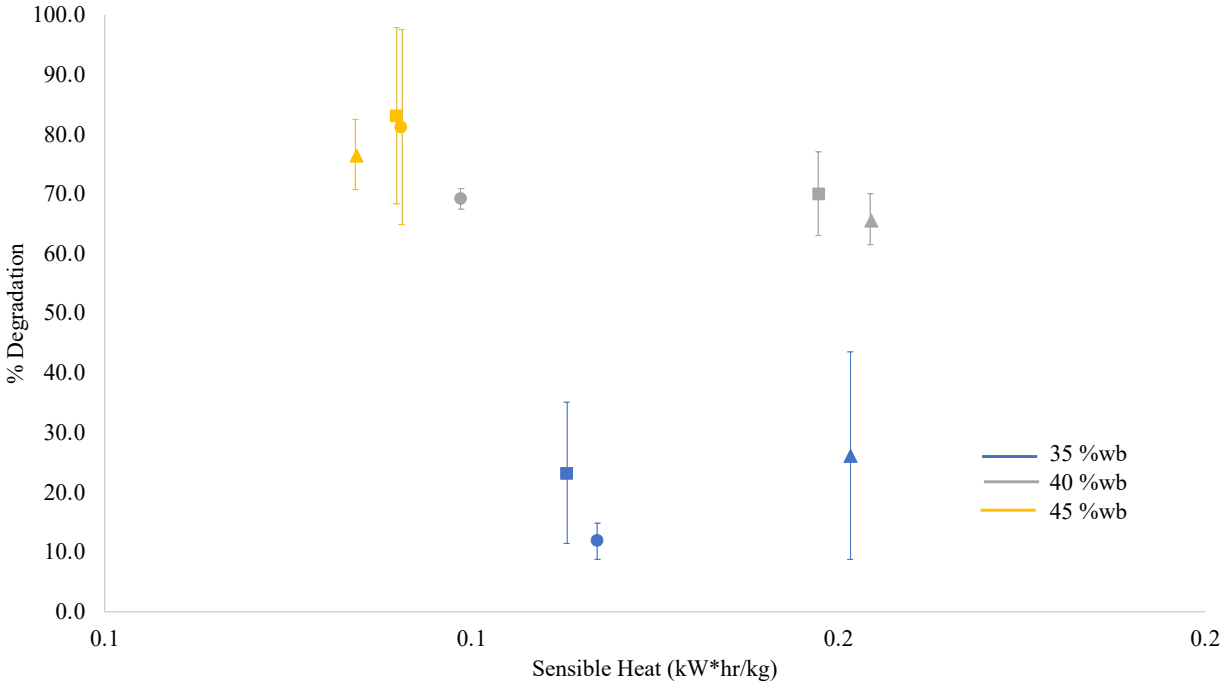

Figure 5.8: Total percent aflatoxin degradation versus sensible heat energy.

A comparison of the total percent aflatoxin degradation to shear rate inside the extruder also correlates with the aforementioned results. Figure 5.9 shows total percent degradation as a function of both effective and weighted shear stress as calculated in Section 5.3.3. The results in Figure 5.9 agree with the results seen in Figure 5.7, where moisture content is again the dominating factor in degradation, rather than the inputted energy. Again, higher degradation is seen at higher moisture contents, which correlate with longer residence times.

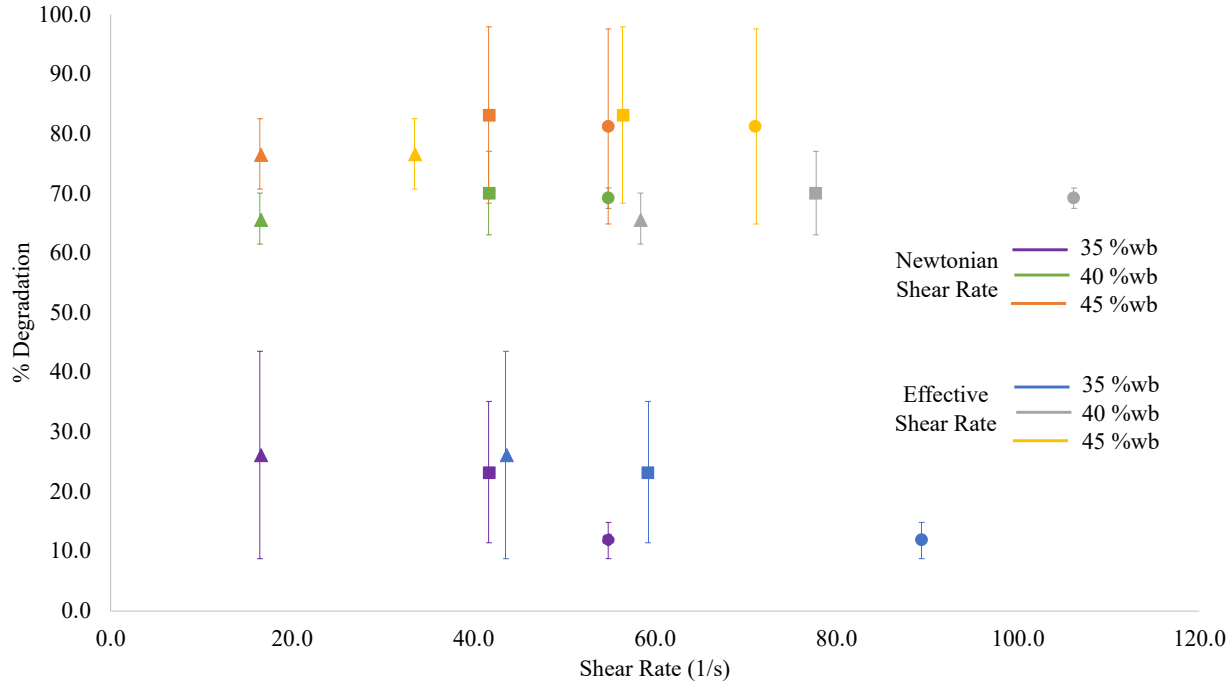

Figure 5.9: Total aflatoxin percent degradation versus effective and Newtonian shear rate.

Shear stress is a function of shear rate and viscosity. As such, as either shear rate or viscosity increases, shear stress also increases. In extrusion processing, the shear rate is also related to the speed at which the material passes through the extruder (e.g. flow rate). The effect of shear stress on aflatoxin degradation inside the extruder can be further examined by evaluating the relationship between moisture content, shear stress, and total degradation as seen in Figure 5.10. Figure 5.10 shows that total aflatoxin percent degradation decreases with increasing shear stress, which contradicts the expected results from other studies and models for shear degradation of molecules inside extruders (Zhao et al., 2011). The results in Figure 5.10 demonstrate again that, rather than a high shear stress, the residence time and moisture content are again the dominating

factor in the amount of aflatoxin degraded. It is reiterated that moisture content is of critical concern when seeking to use extrusion as a reprocessing method for aflatoxin contaminated foods.

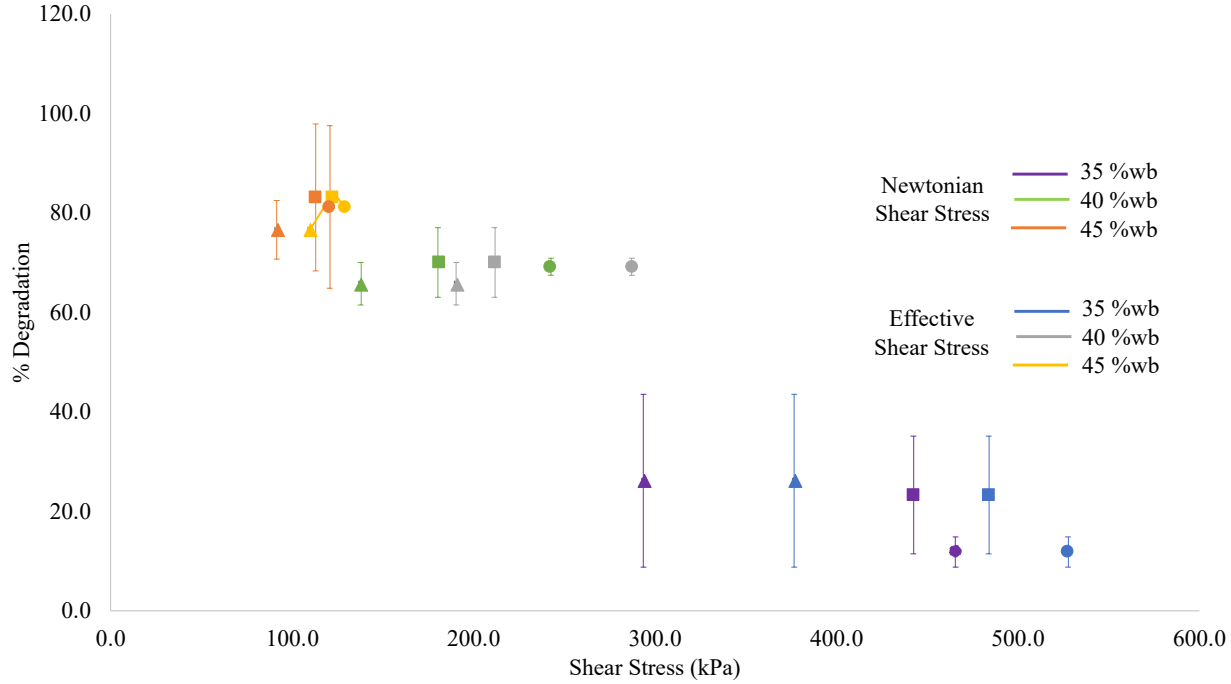

Figure 5.10: Total aflatoxin percent degradation versus shear stress.

The results seen in Figures 5.8, 5.9, and 5.10 are immediately counterintuitive. It might be expected that, even at short residence times, a higher shear rates would result in more aflatoxin degradation in comparison to products with low shear rates and longer residence times. This may be true for larger, industrial extruders, which are able to apply large amounts of shear force to a product in a small amount of times. However, similar results regarding the significance of moisture and screw speed (and thus, shear stress) have been recorded when determining the effect of extrusion on aflatoxin. Zheng et al found that while moisture and barrel temperature were significant, screw speed was not. However, feed rate was also not significant in this study using peanuts (Zheng et al., 2015).

The confounding results regarding aflatoxin degradation and shear stress are reiterated when attempting to apply the Basedow Model to the extrusion data as seen in Section 5.3.4. The nonlinear relationship between shear forces and aflatoxin degradation makes kinetic modeling of shear effects on molecule degradation difficult. Further examination of the results seen in Figure 5.5 demonstrate a trend similar to an enzymatic reaction, where a minimum temperature is

necessary to help catalyze the reaction, but too high of temperatures render the enzyme inactive. Comparing the shear kinetics with enzyme kinetics, one can equate the effect of temperature on enzyme reaction rate to the effect of moisture on the rate of aflatoxin degradation during extrusion. This analogy between shear effects on aflatoxin reaction kinetics and enzyme kinetics is more clearly seen by the left-skewed curve in Figure 5.11.

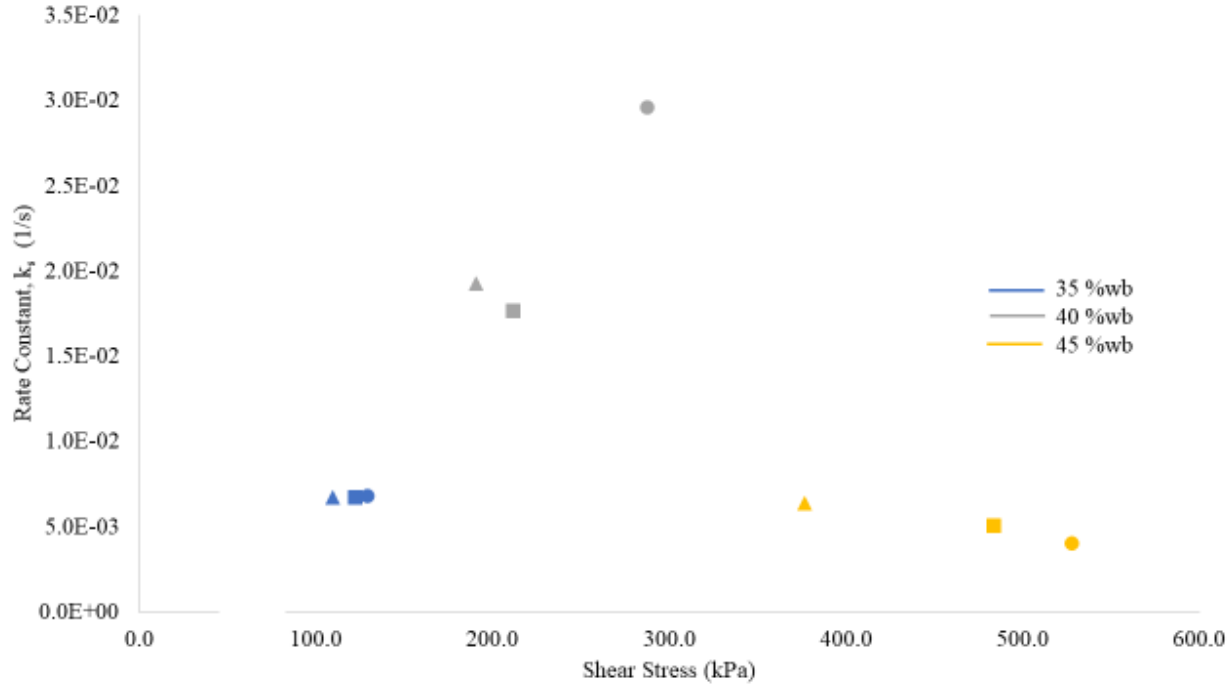

Figure 5.11: Shear Rate Constant (k_s) as a function of shear stress.

It is helpful to view shear stress as a function of moisture content when understanding the role of shear stress on aflatoxin degradation during extrusion. Degradation of aflatoxin becomes increasingly difficult at low moisture contents during physical processing (Doyle et al., 1982). This is likely because moisture content plays a critical role in hydrolyzing the lactone ring structure of aflatoxin, which is important for efficient degradation (Samarajeewa et al., 1990). However, at high moisture content values, Ponrajan (2016) demonstrates that viscosity of the maize product drastically decreases, resulting in a lower shear forces applies to the product. Thus, there is an optimal moisture content that results in maximum aflatoxin degradation when processing contaminated maize via small-scale extrusion. The presence of the potential optimum moisture content is demonstrated in Figure 5.12, where the shear reaction rate constant, k_s, is graphed as a function of moisture content. As seen in Figure 5.11, peak reaction rate occurs at mid-range

moisture contents of approximately 40%. This indicates that contaminated maize flour should be process at or near 40% moisture content for maximum aflatoxin degradation during extrusion at varying motor frequencies.

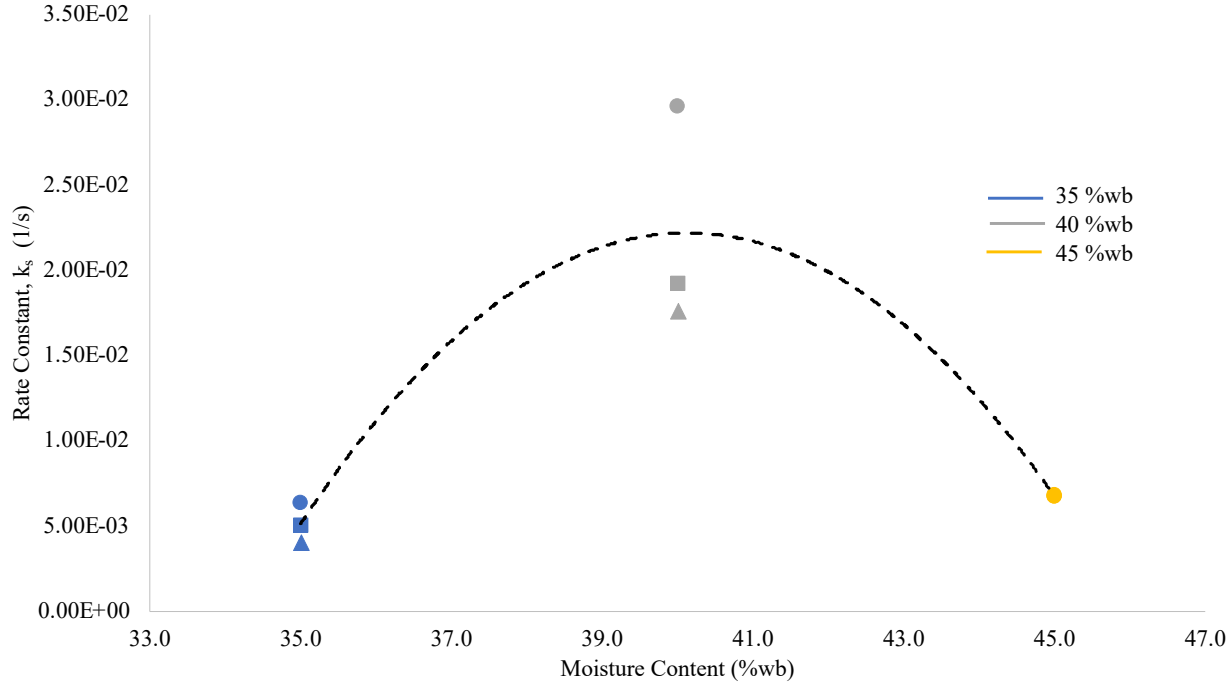

Figure 5.12: Shear Rate Constant (k_s) as a function of product moisture content.

5.5 Conclusions

At high-moisture (35 – 45 %wb) and varying shear rates, small-scale extrusion lacks the necessary temperature increase to thermally degrade aflatoxin in maize products. As such, any degradation that occurs under such conditions can be reasonably attributed to shear forces alone. Determining the exact amount of shear inside the extruder is difficult, given its complex geometry, but a reasonable estimate using effective shear rates and viscosities can be helpful in understanding how shear stress is related to aflatoxin degradation.

In the Mini-Extruder™, high shear rates resulted in lower amounts of degradation because of decreased residence times. These results also indicate that the role of moisture content in the amount of shear applied should not be ignored. As moisture content increases, the residence time increases and higher degradation (>70%) is seen in maize products at a variety of shear rates. Relating moisture content to reaction rate indicates that moisture content acts as a limiting reactant in the degradation of aflatoxin. Thus, the degradation of aflatoxin during extrusion behaves as a time dependent reaction with shear and water acting as a reactant. When water is limiting, degradation cannot occur. However, too much moisture content and the shear stress is insufficient to efficiently achieve a maximum rate of degradation. In cases where water is sufficient, increasing shear would result in higher levels of degradation and revalidate the application of the Basedow model and other mechanochemistry models for the effects of shear on molecules.

Modeling the effects of aflatoxin inside the extruder requires an equation that relates moisture content to the degradation of aflatoxin as both a reactant and an essential factor in predicting shear stress. Still, food processing engineers and extrusion manufacturers should consider operating conditions and extruder designs where product residence time and moisture content are optimized. Moisture content is easily controlled during product preparation prior to extrusion through the addition or subtraction of water. Residence time may be increased through the introduction of longer barrel lengths, decreased feed rates, or increased product viscosity via thickening agents.

Ultimately, there is a complex relationship between varying parameters including moisture content, residence time, screw speed, shear forces, shear rate, and initial concentration when evaluating the effects of extrusion on aflatoxin degradation. The geometry and size of the extruder are also important to consider. As such, modeling the effects of aflatoxin inside the extruder requires an equation that better relates residence time and moisture content to the degradation of

aflatoxin. Studies where residence time remains constant at varying shear rates may confirm that more degradation occurs with greater shear forces. Additionally, studies where moisture content is varied, but residence times remain constant would also be of value for further identifying the effects of moisture content on aflatoxin degradation during extrusion.

6. CONCLUSIONS

Aflatoxin contamination is a pervasive issue across many countries and various food products. As with all research, the search for viable aflatoxin decontamination methods – particularly those in developing countries remains of key concern as the world's population continues to grow towards the year 2050. This study had three main objectives: (1) To conduct a case study in Uasin Gishu County, Kenya, determine the extent of aflatoxin contamination in maize samples in this area, and identify the applicability of extrusion reprocessing technology in the maize supply chain; (2) To determine if small-scale extrusion using the mini-Extruder™ was a viable method for reprocessing aflatoxin-contaminated maize. And, if so, what parameters allows for the highest total percent degradation; and (3) To understand the contribution of shear forces to aflatoxin degradation in maize and make design recommendations for future small-scale extruder designs.

From this work, Uasin Gishu was identified as a county that struggles less with aflatoxin contamination in maize than surrounding counties with lower elevation and more humid conditions. In Trial 1 and Trial 2, only 5% of samples had unsafe levels of aflatoxin (> 10 ppb). However, while these low percentages are promising indicators for the effectiveness of aflatoxin mitigation and education in Uasin Gishu, caution should still be taken when working with such a highly-carcinogenic toxin. Additionally, given Kenya's history of aflatoxin contamination, larger studies (>100 samples) with ample resources for aflatoxin testing are necessary to fully understand the extent of contamination in any region. Still, given the low rates of contamination demonstrated in this study, Uasin Gishu may serve as a viable control region for future aflatoxin mitigation studies in Kenya or East Africa at large.

Storage length was a significant variable in understanding aflatoxin levels in maize samples from across the county. Longer lengths of storage correlated with lower levels in aflatoxin, which is initially counterintuitive given the understanding of crop storage and post-harvest loss. However, when evaluating length of storage as a proxy for net wealth – especially among farmers – it aligns well with the results that farmers with larger amounts of land and thus more stored maize, are able better able to afford aflatoxin mitigation technologies and practices. Additionally, the agricultural conditions under which this field work was conducted were particularly wet and thus, samples

collected closer to their harvest date were more subject to high levels of moisture and poor growing conditions, which promote aflatoxin production in maize.

Environmental conditions (e.g. high temperature and moisture content) are still of great concern in understanding how to mitigate aflatoxin risk. Processing companies and traders in particular, especially those who source their maize from outside the county, should employ strict screening techniques to ensure low levels of aflatoxin in product that are grown under humid and warm conditions. Unfortunately, aflatoxin testing remains largely too expensive for the average trader in local communities and is highly unregulated at the commercial level. Reprocessing methods like small-scale extrusion may help as an additional preventative reprocessing method that ensures greater food safety, but also remains largely too expensive for most entrepreneurs, institutions, and non-governmental organizations. A focus on preventative methods, rather than reactive methods (e.g. reprocessing) may be more effective in developing countries like Kenya where aflatoxin exposure is significantly high because of poor growing conditions and lack of financial access to aflatoxin mitigation technologies and education.

Upon the development of more affordable designs, the inclusion of small-scale extrusion processing in small and medium scale food processing enterprises may help to mitigate aflatoxin. The complexity of the food supply chain in Kenya demands checks and balances in place along each step in the value chain, including the processing level. Small-scale extrusion provides an additional safety and control step in the food supply chain, particularly for at-risk crops like maize. As researchers seek to find viable methods for aflatoxin contamination, they should consider extruders and design them with the effects of shear optimized in mind for the greatest decrease in aflatoxin levels. However, more work to determine the processing conditions needed for consistent aflatoxin degradation is necessary before considering extrusion a viable reprocessing method.

From a technical perspective, small-scale extrusion has the ability to reduce aflatoxin in contaminated maize flour. Small-scale extrusion at varying motors speeds (15 – 50 hz) and moisture content (35 – 45 %wb) can achieve total percent degradation between 11 and 83 % for aflatoxins in maize flour. Optimal aflatoxin degradation occurs at 43 %wb and 45 hz. Moisture content plays a significant role in total aflatoxin degradation (p-value < 0.05) as well as influences maximum temperature, feed rate, viscosity, and residence time inside the extruder. Higher moisture contents (e.g. 45 %wb) contribute to increased viscosity and thus a longer residence time inside the extruder, increasing the time the product is exposed to shear forces during processing.

At high-moisture (35 – 45 %wb) and varying shear rates, small-scale extrusion lacks the necessary temperature increase to thermally degrade aflatoxin in maize products. As such, any degradation that occurs under such conditions can be reasonably attributed to shear forces alone. Determining the exact amount of shear inside the extruder is difficult, given its complex geometry, but a reasonable estimate using effective shear rates and viscosities can be helpful in understanding how shear effects aflatoxin degradation. In the Mini-Extruder™, high shear rates resulted in lower amounts of degradation because of decreased residence times. These results are largely counterintuitive, given that most predictive models for shear degradation of molecules correlates larger shear rates (and thus shear forces) with increased degradation. However, it seems that the role of residence time in these models is largely unaddressed, but plays an important role in determining overall degradation of aflatoxin. Increased residence times, which are a function of viscosity and flow rate, also reiterates that the role of moisture content in molecule degradation during extrusion. The results from this study indicate that residence time and moisture content, rather the magnitude of shear force applied, are critical in degrading aflatoxin via extrusion processing. As such, food processing engineers and extrusion manufacturers should consider operating conditions and extruder designs where residence time inside the extruder is optimized. This may be through the introduction of longer barrel lengths, removing shear bushings from the extruder, decreased feed rates, or increased product viscosity (e.g. higher moisture content).

When evaluating the effects of extrusion on aflatoxin degradation, there is a complex relationship between varying parameters including moisture content, residence time, screw speed, shear forces, shear rate, and initial aflatoxin concentration. The geometry and size of the extruder are also important to consider. As such, modeling the effects of aflatoxin inside the extruder requires an equation that better relates residence time to the degradation of aflatoxin and requires a more complex understanding of degradation due to shear forces along the length of the extruder. Studies where residence time remains constant at varying shear rates may confirm that more degradation occurs with greater shear forces. Additionally, studies where moisture content is varied, but residence times remain constant would also be of value for further identifying the effects of moisture content on aflatoxin degradation during extrusion.

7. FUTURE WORK

As the world's population continues to increase towards the year 2050, reducing the risk of aflatoxin in staple crops like maize will be critical to ensuring food security and public health, especially in developing countries like Kenya. Local field studies such as the one completed for this study are only a small piece of the larger challenges experienced by subsistence farmers and low-income communities regarding aflatoxin contamination around the globe. While maize in Uasin Gishu seems to be relatively unaffected by aflatoxin compared to neighboring counties and other regions in Kenya, more efforts to understand how Kenyans are exposed to aflatoxin must be studied and identified. This includes a better understanding of the link between sourcing and aflatoxin levels, creating more affordable aflatoxin tests, and identifying other at-risk crops.

One highly at-risk food product for aflatoxin are groundnuts (peanuts). As a crop that grows in the ground, peanuts are at risk for aflatoxin contamination via high moisture levels and via exposure to contaminated soil. During the course of this study, 75 samples groundnuts were collected from street vendors ("hawkers") and local grocery store chains in addition to maize samples from around the county. Following collection, all samples were milled and analyzed in duplicate for aflatoxin levels. The findings for aflatoxin levels in groundnuts were noticeably higher than those in the maize samples and confirmed current available research regarding the high risk of aflatoxin exposure when consuming peanuts (Jalili, 2015; Ndisio, 2015).

20% of groundnut samples tested positive for aflatoxin levels above 10 ppb. Additionally, 12% of samples tested positive for levels above 20 ppb. There was a significant correlation ($p < 0.05$) between aflatoxin levels from the open-air market and those purchased in the store. The average level of aflatoxin in samples from the open-air market was 11.3 ppb. Similar to maize, the challenge with groundnuts purchased from the open-air market is the lack of tracing – it is difficult to identify the source of contaminated ground nuts. It is recommended that consumers avoid purchasing groundnuts from open air markets, particularly hawkers whose groundnut supply comes from an unknown origin and likely receives no quality inspection before sale. The high levels of aflatoxin contamination in peanuts in comparison to maize samples indicate that future work on reprocessing peanuts may be more critical to ensuring public health than reprocessing methods for maize.

Future work using small-scale extruders like the Mini-Extruder™ will be of continued importance as economies grow in middle-income countries like Kenya and expand their agricultural sectors. However, the cost of such extrusion technology still remains largely inaccessible The Mini-Extruder™ is approximately ~$20,000 USD. With an average annual income of $3,500, most Kenyan food entrepreneurs would be unable to afford such a technology without the significant aid or a large business loan. Complex financial structures and lack of economic infrastructure make this particularly difficult in rural communities where such technology would be of greatest benefit.

Ideally, steps would also be taken to start manufacturing the extruder in country to reduce the costs associated with international trade of such complex and heavy equipment. This includes more work to simplify and streamline the extruder design – including advanced rheological and design modeling to reduce the cost of the processing equipment. Many attempts were made to determine a model that accurate represented the effect of moisture content of aflatoxin degradation inside the extruder. Such a relationship between aflatoxin degradation and moisture content can be evaluated in two ways. First, moisture content is considered a reactant for aflatoxin degradation as seen in Equation 31.

$$Rate = k[A]^m[MC]^n \tag{31}$$

Where A is the molar concentration of aflatoxin, MC is the molar concentration of water, m and n represent the order of the reaction, and k is the reaction rate constant in 1/s. In this scenario, water can behave as limiting reactant for aflatoxin degradation under low moisture conditions.

A similar model in including moisture content in the degradation equation was developed by Liu et al (2018). Liu et al assumed a pseudo first order reaction for the degradation of aflatoxin in their study on aflatoxin B1 degradation using electron beam irradiation. As such, Liu et al's pseudo first-order equation is summarized seen Equation 32.

$$\frac{C_t}{C_0} = exp(-(A + B * Dose)) \tag{32}$$

Where C_t is the concentration at time t, C_0 is the initial concentration, t is the processing time, A and B are the pseudo first-order rate constants, and dose is the initial concentration of aflatoxin. Using this model, Liu et al discovered that the initial aflatoxin concentration played a role in aflatoxin reduction, which should be taken into consideration in future studies using extrusion.

Additionally, Liu et al discovered that moisture content significant change the rate constant, k, similar to the results in this study seen in Section 5.3 where the shear reaction rate k. As seen in Figure 5.11, the rate constant seems to peak at an optimal moisture content around 40 %wb. While Equations 31 and 32 address the role of moisture in limiting the aflatoxin degradation reaction as a reactant, they fail to account for the effect of moisture content on the actual rate constant k. In the case of the extruder, it is predicted that the rate constant, k, is a function of shear stress, temperature, and moisture content as seen in Equation 33.

$$k = k_0 * \mathrm{f}\big(\mathrm{T}(\mathrm{MC}, \dot{\gamma}), \tau\big(\mathrm{MC}, \dot{\gamma}, \eta(\mathrm{MC}, \mathrm{T})\big), \mathrm{MC}\big) \tag{33}$$

Where T is temperature as a function of shear rate, $\dot{\gamma}$, and moisture content, τ is shear stress as a function of moisture content, shear rate, and viscosity, and MC is moisture content. Equation 33 behaves as a modified version of the Arrhenius equation that may be similar to enzyme kinetics. During enzymatic reactions, the enzyme activity and the maximum reaction rate are highly dependent on the temperature at which the reaction is taking place. At temperatures that are too low, there is insufficient energy to activate the enzyme and catalyze the reaction. At too high of temperatures, the enzyme is denatured and the reaction rate drastically reduces. Thus, there is an optimal temperature range under which a particular reaction occurs. It is predicted that this same behavior is true for aflatoxin degradation during small-scale extrusion. In the same way enzyme-catalyzed reactions are dependent on an optimized temperature, aflatoxin degradation is dependent on an optimized moisture content.

Future work should consider the dual role that moisture content can play in aflatoxin degradation during extrusion as both a limiting reactant and a variable that effects the shear degradation inside the extruder. It would be of great value to discover ways to test aflatoxin decontamination at a constant moisture content while varying screw speeds (i.e. shear rates) and temperatures to empirically derives the relationship written in Equation 33.

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APPENDIX A. SCATTER PLOTS

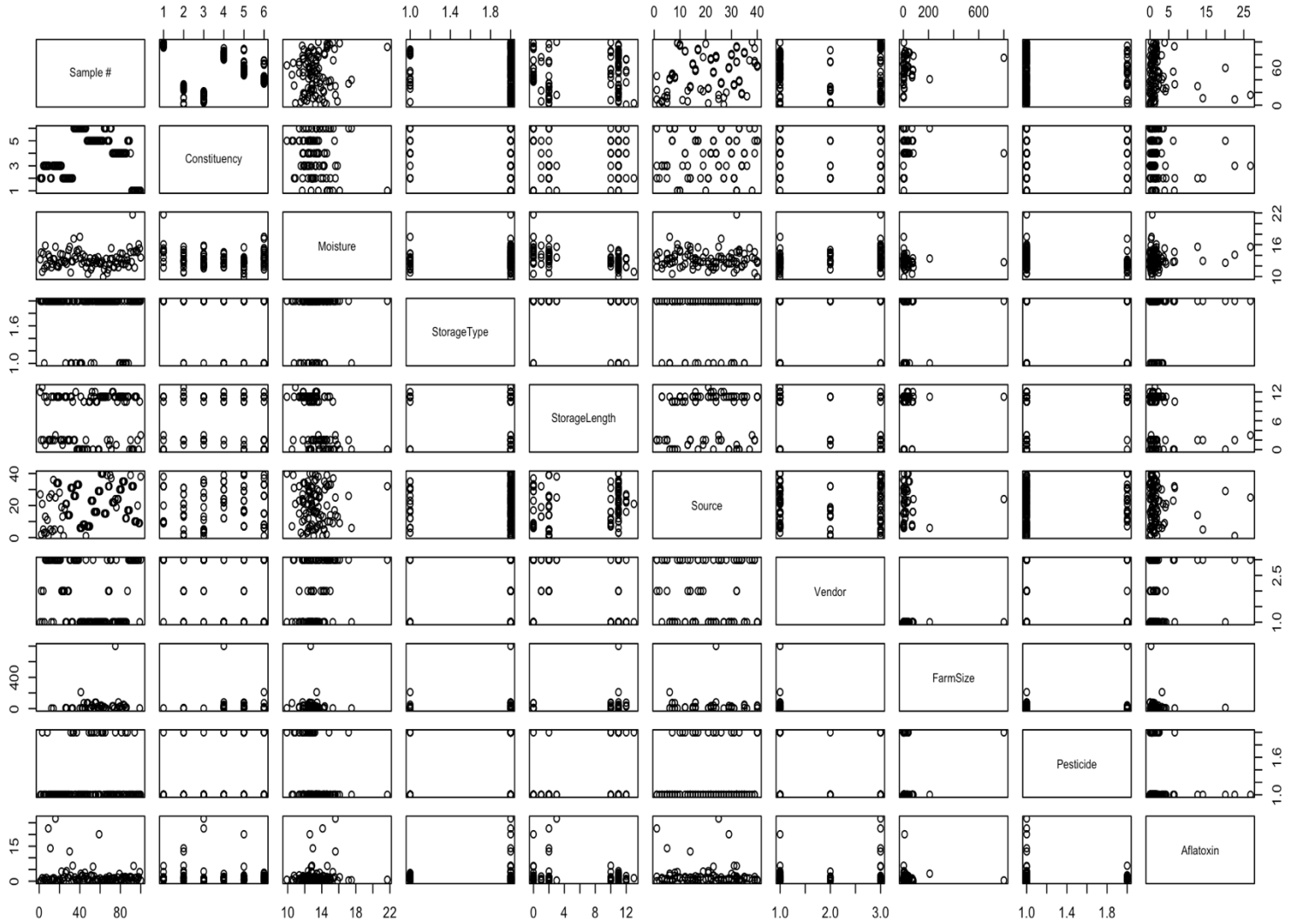

Figure A1: Trial 1 scatter plot comparing all variables.

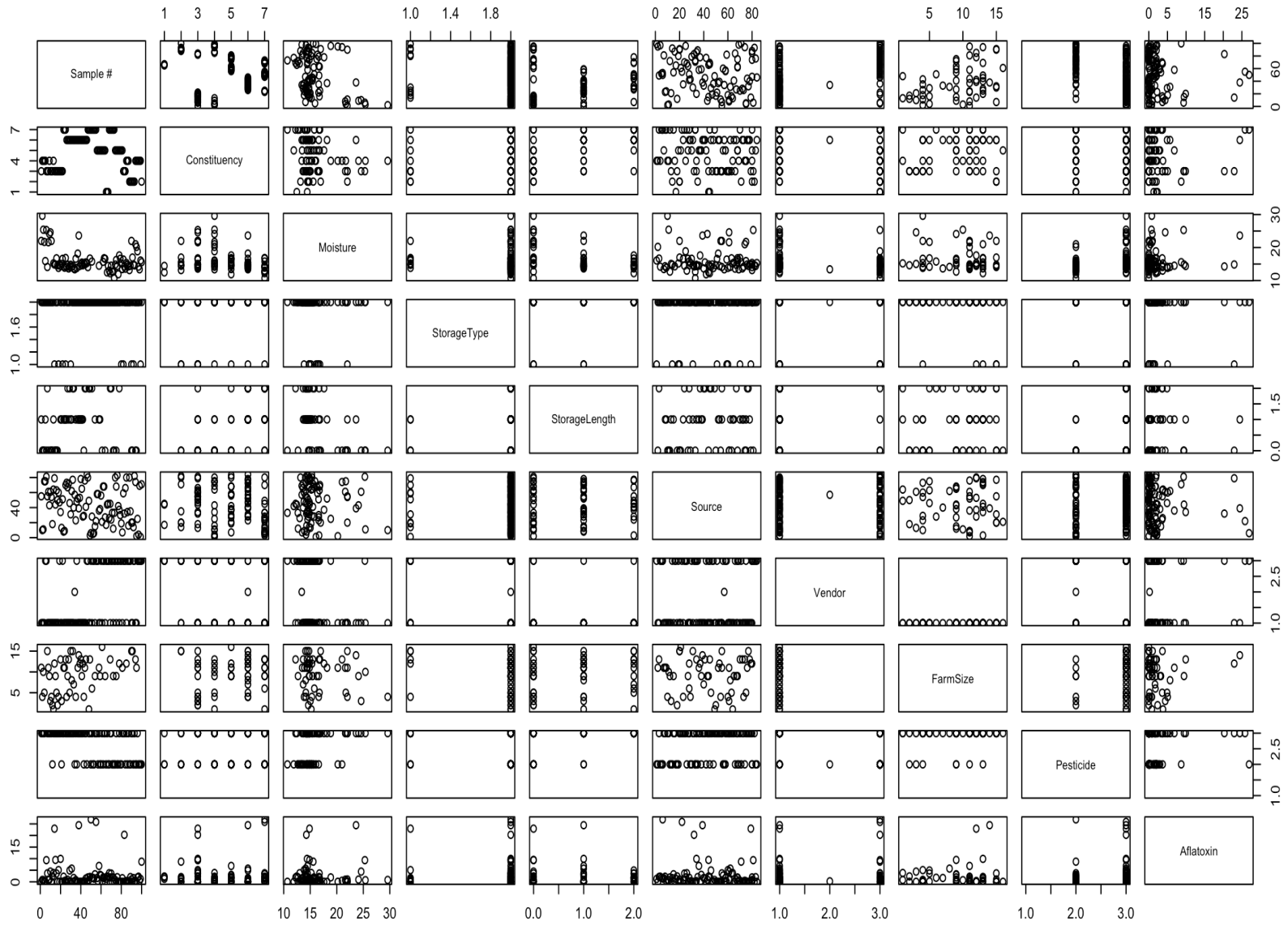

Figure A2: Trial 2 scatter plot comparing all variables.

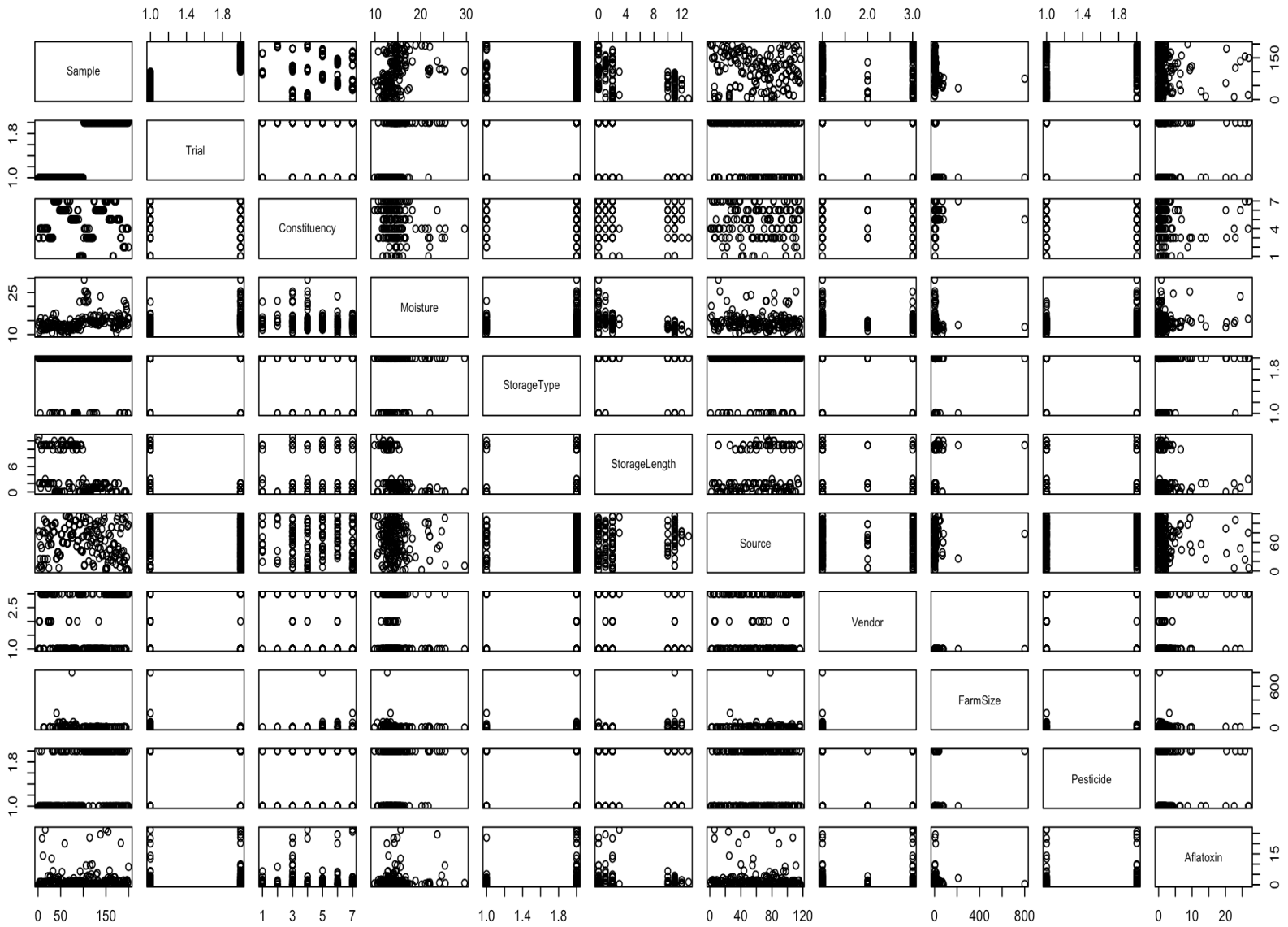

Figure A3: Scatter plots comparing Trial 1 and Trial 2

APPENDIX B. FIELD DATA

Sample #	Constituency	Moisture (%wb)	Storage Type	Storage Length (months)	Source	Vendor	Farm Size (acres)	Pesticide	Aflatoxin (ppb)
1	Kapseret	13.3	Non_Hermetic	12	Ngeria	Farmer		No	0
2	Kapseret	14.6	Non_Hermetic	2	Busia County, Western	Poshomori		No	0.2
3	Kapseret	10.9	Non_Hermetic	13	Megun	Farmer		Yes	1.3
4	Kesses	14.3	Non_Hermetic	2	Kakamega	Poshomori		No	0
5	Kesses	11.6	Hermetic	11	Cheptiret	Farmer		No	1
6	Kesses	15.9	Non_Hermetic	1	Kipkaren	Trader		No	0.2
7	Kesses	12.3	Non_Hermetic	11	Cheptiret	Trader		No	1.4
8	Kesses	12.0	Non_Hermetic	10	Kesses	Trader		Yes	0.4
9	Kesses	14.1	Non_Hermetic	2	Bungoma	Trader		No	22.6
10	Kesses	13.7	Non_Hermetic	2	ndi	Trader		No	0.7
11	Kapseret	13.0	Non_Hermetic	2	Kakamega	Trader		No	14.1
12	Kapseret	11.8	Non_Hermetic	11	Ngeria	Farmer	2	No	0.7
13	Kesses	12.4	Non_Hermetic	2	Cheptiret (Kakamega)	Trader		No	0.8
14	Kesses	13.3	Non_Hermetic	11	Tulwet/Chuiyat	Farmer	1	No	1.3
15	Kesses	13.3	Non_Hermetic	2	Kakamega	Trader		No	1.1
16	Kesses	15.6	Non_Hermetic	3	ndi	Trader		No	26.8
17	Kesses	12.0	Non_Hermetic	11	Tarakwa	Trader		No	0.3
18	Kesses	12.0	Non_Hermetic	11	Tarakwa	Trader		No	0
19	Kesses	11.9	Non_Hermetic	11	Tarakwa	Trader		No	0
20	Kesses	12.6	Non_Hermetic	11	Racecourse	Trader		No	0.7
21	Kesses	14.3	Non_Hermetic	2	Kakamega	Trader		No	0.1
22	Kesses	12.8	Non_Hermetic	2	Marakwet	Poshomori		No	4.1
23	Kapseret	14.2	Non_Hermetic	1	Kipkaren	Poshomori		No	1
24	Kapseret	13.1	Non_Hermetic	2	Bungoma	Poshomori		No	0
25	Kapseret	12.7	Non_Hermetic	11	Langas	Poshomori		No	1.8

26	Kapseret	12.9	Hermetic	11	Megun	Farmer	8	No	3.5
27	Kapseret	12.4	Non_Hermetic	11	Megun	Farmer	5	No	4.3
28	Kapseret	13.9	Non_Hermetic	2	Kipkenyo	Poshomori		No	1.5
29	Kapseret	15.0	Non_Hermetic	2	Kipkenyo	Poshomori		No	1.5
30	Kapseret	15.6	Non_Hermetic	2	Kipkenyo	Trader		No	12.7
31	Kapseret	10.8	Hermetic	11	Simat	Trader		Yes	2.3
32	Kapseret	12.9	Hermetic	11	Simat	Farmer	2	No	0
33	Kapseret	12.9	Non_Hermetic	10	Simat	Farmer	3	Yes	6.6
34	Turbo	17.2	Non_Hermetic	2	Ngenyilel	Trader		Yes	0.3
35	Turbo	13.8	Non_Hermetic	2	Ngenyilel	Trader		No	1.6
36	Turbo	13.4	Hermetic	12	Ngenyilel	Trader		No	0.6
37	Turbo	14.9	Non_Hermetic	0	Tapsagoi	Trader		Yes	0
38	Turbo	14.5	Non_Hermetic	0	Tapsagoi	Trader		No	0
39	Turbo	15.1	Non_Hermetic	0	Tapsagoi	Trader		No	0.8
40	Turbo	17.5	Hermetic	0	Kamagut	Farmer	3	No	0.4
41	Turbo	13.4	Hermetic	11	Kamagut	Farmer	210	No	3.2
42	Turbo	12.5	Hermetic	0	Kamagut	Farmer	5.6	No	2.4
43	Turbo	14.2	Non_Hermetic	0	Kapsaos	Farmer	2.5	No	2.3
44	Turbo	12.9	Non_Hermetic	10	Kapsaos	Farmer	70	No	1.2
45	Turbo	13.8	Non_Hermetic	0	Kapsaos	Farmer	4	No	3.6
46	Turbo	11.8	Non_Hermetic	2	Bungoma	Trader		No	1.9
47	Soy	12.7	Non_Hermetic	0	Kapkures	Farmer	70	No	0.8
48	Soy	10.5	Non_Hermetic	0	Kapkures	Farmer	70	No	0.6
49	Soy	12.7	Non_Hermetic	10	Kapkures	Farmer	2	Yes	0.5
50	Soy	13.5	Non_Hermetic	10	Kapkures	Farmer	1	No	0.1
51	Soy	11.4	Hermetic	11	Moi's Bridge	Farmer	10	Yes	2.5
52	Soy	11.8	Non_Hermetic	12	Moi's Bridge	Farmer	40	Yes	2.2
53	Soy	12.8	Non_Hermetic	0	Moi's Bridge	Trader		No	6.2
54	Soy	13.2	Hermetic	11	Kipsombe	Farmer	7	Yes	2.1
55	Soy	13.5	Non_Hermetic	12	Kipsombe	Farmer	76	No	0.7
56	Soy	12.6	Non_Hermetic	10	Kipsombe	Farmer	76	No	0
57	Soy	13.0	Non_Hermetic	10	Kipsombe	Farmer	20	Yes	0.7
58	Soy	13.7	Non_Hermetic	0	Segero	Farmer	5	No	0.7
59	Soy	12.6	Non_Hermetic	0	Segero	Farmer	10	No	20.1
60	Soy	12.5	Non_Hermetic	11	Segero	Farmer	34	No	1.2
61	Soy	12.6	Non_Hermetic	11	Ziwa	Farmer	40	Yes	0.4

62	Soy	12.9	Non_Hermetic	11	Ziwa	Farmer	30	Yes	0.3
63	Soy	9.9	Non_Hermetic	11	Ziwa	Farmer	5	Yes	0.6
64	Turbo	11.4	Non_Hermetic	11	Kiplombe	Farmer	20	No	0.1
65	Turbo	12.2	Non_Hermetic	11	Kiplombe	Farmer	0.5	Yes	0.6
66	Turbo	13.4	Non_Hermetic	11	Kiplombe	Farmer	2	No	1.4
67	Soy	10.7	Non_Hermetic	2	Western	Trader		No	0.3
68	Soy	11.4	Non_Hermetic	11	Soy	Poshomori		No	1
69	Soy	12.4	Non_Hermetic	1	Soy	Poshomori		No	2
70	Turbo	13.3	Non_Hermetic	2	Western	Trader		No	0.7
71	Turbo	15.4	Non_Hermetic	0	Uasin Gishu	Trader		No	1.2
72	Moiben	13.4	Non_Hermetic	12	Melbeki	Farmer	30	No	1
73	Moiben	11.9	Non_Hermetic	12	Melbeki	Farmer	30	No	0.8
74	Moiben	11.8	Non_Hermetic	11	Melbeki	Farmer	3	No	1.3
75	Moiben	12.7	Non_Hermetic	11	Moiben	Farmer	800	Yes	0.3
76	Moiben	13.6	Non_Hermetic	1	Marakwet	Trader		No	0.5
77	Moiben	12.5	Non_Hermetic	10	Moiben	Farmer	9	No	1.6
78	Moiben	11.8	Non_Hermetic	11	Moiben	Farmer	80	No	0
79	Moiben	13.5	Hermetic	11	Sergoit	Farmer	20	No	3.1
80	Moiben	14.4	Hermetic	11	Sergoit	Farmer	28	No	0.6
81	Moiben	12.5	Non_Hermetic	11	Sergoit	Farmer	3	Yes	0.9
82	Moiben	14.3	Hermetic	11	Tembelio	Farmer	8	No	1.8
83	Moiben	12.4	Hermetic	11	Tembelio	Farmer	50	No	1
84	Moiben	13.1	Non_Hermetic	10	Tembelio	Farmer	40	No	1.7
85	Moiben	12.4	Hermetic	11	Kimumu	Farmer	15	Yes	1.8
86	Moiben	13.7	Hermetic	10	Kimumu	Farmer	14	No	1.3
87	Soy	13.1	Non_Hermetic	11	Kuinet	Poshomori		Yes	0.8
88	Soy	11.9	Hermetic	11	Kuinet	Trader		No	0.3
89	Soy	15.5	Non_Hermetic	0	Kuinet	Trader		No	1.7
90	Moiben	14.6	Non_Hermetic	2	Western	Trader		No	1.8
91	Ainabkoi	12.8	Non_Hermetic	0	Soy	Trader		No	1.5
92	Ainabkoi	21.7	Non_Hermetic	0	Soy	Trader		No	0.6
93	Ainabkoi	14.7	Non_Hermetic	0	Soy	Trader		No	6.5
94	Ainabkoi	11.7	Non_Hermetic	11	Kapteget	Trader		Yes	1.3
95	Ainabkoi	14.7	Non_Hermetic	11	Kapteget	Trader		No	1.3
96	Ainabkoi	15.0	Non_Hermetic	11	Kapteget	Trader		No	1.5
97	Ainabkoi	14.6	Non_Hermetic	2	Maraquet	Trader		No	0.8
98	Ainabkoi	16.1	Non_Hermetic	0	KapSoya	Trader		No	1.7
99	Ainabkoi	15.3	Non_Hermetic	10	KapSoya	Farmer	4	No	3.9
100	Ainabkoi	13.6	Non_Hermetic	3	Wesern	Trader		No	0.3

Sample #	Constituency	Moisture (%wb)	Storage Type	Storage Length (months)	Source	Vendor	Farm Size (acres)	Pesticide	Aflatoxin (ppb)
1	Kapseret	22.0	Non_Hermetic	1	Megun	Farmer	3	Yes	0.1
2	Kesses	29.6	Non_Hermetic	0	Cheptiret	Farmer	1	Yes	0.8
3	Kesses	25.4	Non_Hermetic	0	Chuiyat	Farmer	2.5	Yes	0.7
4	Kesses	21.7	Non_Hermetic	0	Tarakwa	Farmer	10	Yes	0.15
5	Kesses	15.9	Non_Hermetic	1	Tarakwa	Trader			0
6	Kapseret	25.3	Non_Hermetic	0	Unsure	Trader			9.35
7	Kapseret	14.1	Non_Hermetic	2	Megun	Farmer	8	Yes	3.65
8	Kapseret	21.6	Non_Hermetic	0	Ngeria	Farmer	3	Yes	0.9
9	Kesses	24.1	Non_Hermetic		Kipchamo	Farmer	2	Yes	0.95
10	Kapseret	24.6	Non_Hermetic	0	Ngeria	Farmer	0.5	Yes	4.4
11	Kapseret	22.0	Non_Hermetic	0	megun	Farmer	1	Yes	0
12	Kapseret		Non_Hermetic	0	iriri	Farmer	0.4	No	2.35
13	Kesses	15.2	Non_Hermetic	1	Race course	Farmer	0.3	Yes	3.8
14	Kapseret	14.9	Hermetic	0	Tuiyobei	Farmer	4	Yes	22.95
15	Kapseret	14.9	Non_Hermetic	0	kipkenyo	Farmer	2	Yes	9.45
16	Kapseret	16.7	Non_Hermetic	0	nyamumbi	Farmer	10	Yes	3.6
17	Kapseret	14.6	Non_Hermetic	0	langas	Farmer	0.4	Yes	0
18	Kapseret	13.8	Hermetic		near kimare	Farmer	1	Yes	5.05
19	Kapseret	14.9			Kitale	Trader		Yes	0
20	Kapseret	14.4	Non_Hermetic	1	Kapseret	Farmer	5	Yes	9.9
21	Kapseret	15.0	Non_Hermetic	1	estate sai	Farmer	0.5	No	0
22	Kapseret	16.6	Hermetic		local farmers	Trader		Yes	0
23	Turbo	14.3	Non_Hermetic	1	chepkongi	Farmer	3	Yes	2.2

24	Turbo	13.9	Non_Hermetic	1	Chepkongi	Farmer	3		0.35
25	Turbo	16.8	Hermetic	1	siriat	Farmer	5	Yes	0.5
26	Soy	14.2	Non_Hermetic	1	Soy dam	Farmer	1	Yes	0
27	Soy	14.8	Non_Hermetic	2	serekea	Farmer	2	Yes	0.9
28	Soy	13.7	Non_Hermetic	1	Soy	Farmer	5	Yes	0.05
29	Soy	14.2	Non_Hermetic	2	kipsomba	Farmer	10	Yes	4.9
30	Soy	14.8	Hermetic	1	Kapndi	Farmer	8	Yes	0
31	Soy	14.1	Non_Hermetic	1	ngili	Farmer	15	Yes	5.7
32	Soy	14.4	Non_Hermetic	2	Tei	Farmer	8	Yes	0
33	Soy	13.7	Non_Hermetic	2	kilima	Farmer	12	Yes	1.65
34	Soy	13.4	Non_Hermetic		moi's bridge	Poshomori		No	0.2
35	Soy	16.6	Non_Hermetic	1	kapchan	Farmer	1	Yes	0.75
36	Soy	13.8	Non_Hermetic		kuinet	Trader		No	0
37	Soy	18.2	Non_Hermetic	1	long'net	Farmer	3	Yes	0
38	Soy	23.6	Non_Hermetic	1	kerotet	Farmer	7	Yes	24.45
39	Soy	14.4	Non_Hermetic	1	makongi	Farmer	4	Yes	0
40	Soy	14.9	Non_Hermetic	1	tekeyat	Farmer	5	Yes	0
41	Soy	15.4	Non_Hermetic	1	segero	Farmer	2	Yes	0
42	Soy	14.6	Non_Hermetic	1	kapkures	Farmer	4	Yes	0
43	Soy	15.3	Non_Hermetic	0	kapkures central	Farmer	1	No	0.25
44	Soy	14.9	Non_Hermetic	2	Tebeson	Farmer	3	Yes	0
45	Soy	15.5	Non_Hermetic	2	kimolwet	Farmer	5	Yes	1.4
46	Soy	15.3	Non_Hermetic		ziwa machine	Trader		No	0
47	Turbo	14.1	Non_Hermetic		Turbo	Trader			1.9
48	Turbo		Non_Hermetic	2	labuiywet	Farmer	0.3	Yes	0.8
49	Turbo	16.7	Non_Hermetic	2	besibor	Farmer	5	Yes	2.1
50	Turbo		Non_Hermetic		Bungoma	Trader		No	26.95
51	Turbo	15.8	Non_Hermetic	2	kapkures	Farmer	11	Yes	1.75
52	Turbo		Non_Hermetic		bukwo	Trader		No	1.7
53	Turbo		Non_Hermetic		Bungoma	Trader		No	1.4
54	Turbo		Non_Hermetic	1	huruma	Farmer	2	Yes	3.25
55	Turbo		Non_Hermetic		kakamega	Trader		Yes	25.85

56	Moiben		Non_Hermetic		Moiben	Trader		Yes	1.65
57	Moiben		Non_Hermetic		Moiben	Trader		No	0.3
58	Moiben	14.5	Non_Hermetic	1	kaptiren	Farmer	3	Yes	6.85
59	Moiben		Non_Hermetic	1	karandili	Farmer	2	No	3
60	Moiben		Non_Hermetic		meibeki	Trader		No	2.7
61	Moiben		Non_Hermetic	0	kabiyet	Farmer	Not sure	Yes	0
62	Moiben	15.7	Non_Hermetic		sergoit	Trader		No	1.85
63	Moiben	16.8	Non_Hermetic		sergoit	Trader		Yes	1.7
64	Moiben	16.6	Non_Hermetic	0	kapkulunga	Farmer	2	Yes	0.1
65	Ainabkoi		Non_Hermetic		ilula	Trader		No	1.3
66	Ainabkoi	12.5	Non_Hermetic		kipkenyo	Trader		Yes	2.3
67	Ainabkoi	14.4	Non_Hermetic		kiplombe	Trader		No	2
68	Turbo	13.0	Non_Hermetic		kapkeben	Trader		No	3.7
69	Turbo	12.2	Non_Hermetic	2	kiplombe	Farmer	5	Yes	1.9
70	Turbo	14.5	Non_Hermetic	2	kapchumba	Trader		Yes	3.55
71	Turbo	12.7	Non_Hermetic		kapsaos	Trader		No	0
72	Turbo	12.7	Non_Hermetic	0	emkwen	Farmer	2	No	1.9
73	Turbo	10.7	Non_Hermetic	0	kapsaos	Trader		No	0
74	Moiben	14.8	Non_Hermetic		Turbo	Trader		No	0.8
75	Moiben	16.2	Non_Hermetic	0	sergoit	Farmer	2	Yes	0
76	Moiben	14.4	Non_Hermetic		ziwa	Trader		No	2
77	Moiben	11.9	Non_Hermetic		kimumu	Trader		No	0
78	Moiben	17.6	Non_Hermetic	2	kaptuli	Farmer	4	Yes	0
79	Moiben	16.6	Non_Hermetic		kaplogoi	Trader		No	0.1
80	Moiben	15.2	Hermetic		iten	Trader		No	0
81	Moiben	13.5	Non_Hermetic		chembulet	Farmer	3	Yes	1.55
82	Kapseret	16.4	Hermetic		mugundoi	Trader		Yes	0
83	Kapseret	14.3	Non_Hermetic		kapsabet	Trader		Yes	20.3
84	Kapseret	13.6	Non_Hermetic		sellier	Trader		No	0
85	Kesses	15.3	Non_Hermetic		Turbo	Farmer	4	Yes	0.35
86	Kesses	14.8	Non_Hermetic		Kapseret	Trader		No	0.3
87	Kesses	14.3	Non_Hermetic		iberi	Trader		Yes	0
88	Ainabkoi	13.2	Non_Hermetic		Turbo	Trader		No	0

89	Ainabkoi	14.5	Non_Hermetic		kaptagat	Trader		No	2.05
90	Ainabkoi	22.0	Hermetic	0	kabirmei	Farmer	8	Yes	1
91	Ainabkoi	17.0	Non_Hermetic	0	kabirmei	Farmer	8	Yes	0
92	Ainabkoi	14.8	Hermetic		flax	Trader		No	1.6
93	Ainabkoi	14.6	Non_Hermetic		wanifor	Trader		Yes	0
94	Kesses	21.0	Non_Hermetic	0	tarakwa	Farmer	5	No	0.45
95	Kesses	20.2	Non_Hermetic	0	bayete	Farmer	3	No	0
96	Kesses	18.9	Non_Hermetic		kapg'etuny	Trader		Yes	1.9
97	Kesses	14.2	Non_Hermetic		bindura	Trader		No	2.2
98	Kesses	13.4	Non_Hermetic		sigilai	Trader		No	0.45
99	Kesses	16.1	Hermetic		annex	Trader		No	1.25
100	Ainabkoi	15.6	Non_Hermetic		sokoni	Trader		No	8.7

APPENDIX C. EXTRUSION TESTING RAW DATA

Operating Conditions			*Aflatoxin % Decrease*			
Motor Frequency (Hz)	**Moisture Content (% wb)**	**Initial Aflatoxin (ppb)**	**Run 1**	**Run 2**	**Run 3**	**Average**
15	35	2.3	29.0	7.5	41.9	26.2
38			31.2	9.7	29.0	23.3
50			*-313.2	14.0	9.7	11.8
15	40	5.8	61.5	65.8	70.1	65.8
38			62.4	76.0	71.8	70.1
50			70.9	67.5	69.2	69.2
15	45	8.7	70.1	81.6	78.2	76.6
38			100.0	77.0	72.4	83.1
50			70.1	73.6	100.0	81.2

**Removed from data analysis after being identified as an outlier. Is also not included in any average calculations.*

Operating Conditions			*Maximum Product Temperature (°C)*			
Motor Frequency (Hz)	**Moisture Content (% wb)**	**Ambient Temperature (°C)**	**Run 1**	**Run 2**	**Run 3**	**Average**
15	35	23.2	83.5	-	-	83.5
38			68.9	-	-	68.9
50			70.5	-	-	70.5
15	40	23.2	86.6	-	-	86.6
38			83.9	-	-	83.9
50			63.0	70.5	60.5	64.7
15	45	23.2	52.8	65.9	61.6	60.1
38			50.1	70.1	67.1	62.4
50			59.2	63.7	65.3	62.7

Operating Conditions		*Product Feed Rate (kg/hr)*			
Motor Frequency (Hz)	**Moisture Content (% wb)**	**Run 1**	**Run 2**	**Run 3**	**Average**
15	35	25.0	-	-	25.0
38		22.6	-	-	22.6
50		38.2	-	-	38.2
15	40	16.4	16.4	31.2	21.3
38		17.4	-	-	17.4
50		22.0	39.3	28.4	29.9
15	45	6.3	4.6	5.6	5.5
38		3.2	5.0	5.2	4.5
50		4.6	5.1	4.9	4.9

Pre-Extrusion Aflatoxin Measurements			
	Moisture Content (% wb)		
Measurement	35	40	45
1	7.6	1.9	1.9
2	2.8	6.0	1.6
3	1.4	1.3	2.3
4	1.7	0.0	2.2
5	1.6	8.3	0.0
6	0.0	0.0	9.5
7	0.0	1.4	22.6
8	0.9	15.1	9.7
9	1.1	28.9	24.6
10	0.0	1.3	5.0
11	8.7	0.1	1.4
12	2.1	-	14.4
13	-	-	25.1
14	-	-	1.1
Average	2.3	5.8	8.7
Std. Dev.	2.9	9.0	9.4

APPENDIX D. STATISTICAL ANALYSIS DATA

Regression Table

Y-hat Model

% decrease aflo

Factor	Name	Coeff	P(2 Tail)	Tol	Active
Const		69.747	0.0000		
A	moisture	27.444	0.0000	0.2698	X
B	speed	1.711	0.5707	0.3378	X
AB		4.844	0.0366	0.9232	X
AA		-16.035	0.0096	0.2610	X
BB		-2.251	0.7022	0.3290	X
AAB		-4.036	0.2869	0.3321	X
ABB		1.513	0.7252	0.2668	X
AABB		-3.508	0.6307	0.1740	X

R^2	0.8720
Adj R^2	0.8481
Std Error	10.3714
F	36.6008
Sig F	0.0000
F_{LOF}	NA
Sig F_{LOF}	NA

Source	SS	df	MS
Regression	31496.1	8	3937.0
Error	4625.3	43	107.6
$Error_{Pure}$	4625.3	43	107.6
$Error_{LOF}$	0.0	0	NA
Total	36121.4	51	

Factor	Name	Low	High	Exper
A	moisture	35	45	35
B	speed	15	50	50

Multiple Response Prediction

	Y-hat	S-hat	99% Confidence Interval Lower Bound	Upper Bound
% decrease aflo	11.8280	2.4832	4.378	19.278

	Moisture	Hz
Best	43	45
Worst	35	50

S-hat Model

% decrease aflo

Factor	Name	Coeff	P(2 Tail)	Tol	Active
Const		7.114	NA		
A	moisture	-0.03792	NA	0.2568	X
B	speed	-1.014	NA	0.3227	X
AB		5.876	NA	0.9529	X
AA		6.010	NA	0.2568	X
BB		-4.227	NA	0.3227	X
AAB		0.26877	NA	0.3210	X
ABB		0.94187	NA	0.2608	X
AABB		1.110	NA	0.1695	X

R^2	1.0000
Adj R^2	NA
Std Error	NA
F	NA
Sig F	NA
F_{LOF}	NA
Sig F_{LOF}	NA

Source	SS	df	MS
Regression	262.7	8	32.8
Error	0.0	0	NA
$Error_{Pure}$	NA	0	NA
$Error_{LOF}$	NA	0	NA
Total	262.7	8	

Effects of Moisture Content and Motor Frequency on Aflatoxin Degradation

Average Aflatoxin % Degradation					
		Motor Frequency (hz)			
	Levels	*1 (15)*	*2 (38)*	*3 (50)*	Mean
Moisture (% wb)	*1 (35)*	25.4	22.5	10.9	19.6
	2 (40)	65.5	69.8	69.0	68.1
	3 (45)	76.6	83.1	81.2	80.3
	Mean	55.8	58.5	53.7	Grand Mean = 56.0

Standard Deviation Aflatoxin % Degradation					
		Motor Frequency (hz)			
	Levels	*1 (15)*	*2 (38)*	*3 (50)*	STDEV
Moisture (% wb)	*1 (35)*	25.4	22.5	10.9	7.7
	2 (40)	65.5	69.8	69.0	2.3
	3 (45)	74.3	81.4	79.3	3.7
	STDEV	26.1	31.2	36.9	27.5

APPENDIX E. THERMAL DEGRADATION OF AFLATOXIN

Extruder Length (m)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9
Moisture (% wb)		*35*			*40*			*45*	
Motor Speed	*15.0*	*38.0*	*50.0*	*15.0*	*38.0*	*50.0*	*15.0*	*38.0*	*50.0*
Extruder Length (m)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**	**Run 7**	**Run 8**	**Run 9**
0	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.00	0.0E+00
0.01	1.3E-11	1.3E-11	8.0E-12	1.6E-11	1.9E-11	9.9E-12	5.2E-11	6.5E-11	6.0E-11
0.02	5.1E-11	4.8E-11	2.9E-11	6.1E-11	7.3E-11	3.5E-11	1.8E-10	2.3E-10	2.1E-10
0.03	1.3E-10	1.2E-10	7.0E-11	1.6E-10	1.9E-10	8.3E-11	4.2E-10	5.4E-10	5.0E-10
0.04	2.8E-10	2.3E-10	1.4E-10	3.4E-10	4.0E-10	1.6E-10	8.2E-10	1.0E-09	9.7E-10
0.05	5.4E-10	4.2E-10	2.6E-10	6.7E-10	7.7E-10	2.9E-10	1.4E-09	1.8E-09	1.7E-09
0.06	9.7E-10	7.2E-10	4.4E-10	1.2E-09	1.4E-09	4.8E-10	2.3E-09	3.0E-09	2.8E-09
0.07	1.7E-09	1.2E-09	7.2E-10	2.1E-09	2.4E-09	7.7E-10	3.6E-09	4.8E-09	4.4E-09
0.08	2.8E-09	1.8E-09	1.1E-09	3.6E-09	4.0E-09	1.2E-09	5.4E-09	7.2E-09	6.7E-09
0.09	4.5E-09	2.7E-09	1.7E-09	5.9E-09	6.5E-09	1.7E-09	7.9E-09	1.1E-08	1.0E-08
0.1	7.0E-09	4.1E-09	2.6E-09	9.4E-09	1.0E-08	2.5E-09	1.1E-08	1.5E-08	1.4E-08
0.11	1.1E-08	5.9E-09	3.8E-09	1.5E-08	1.6E-08	3.6E-09	1.6E-08	2.2E-08	2.0E-08
0.12	1.7E-08	8.5E-09	5.5E-09	2.3E-08	2.4E-08	5.1E-09	2.2E-08	3.0E-08	2.8E-08
0.13	2.5E-08	1.2E-08	7.8E-09	3.5E-08	3.7E-08	7.1E-09	3.0E-08	4.2E-08	3.9E-08
0.14	3.7E-08	1.7E-08	1.1E-08	5.2E-08	5.4E-08	9.8E-09	4.0E-08	5.7E-08	5.4E-08
0.15	5.5E-08	2.3E-08	1.5E-08	7.8E-08	8.0E-08	1.3E-08	5.4E-08	7.7E-08	7.2E-08
0.16	7.9E-08	3.2E-08	2.1E-08	1.1E-07	1.2E-07	1.8E-08	7.2E-08	1.0E-07	9.7E-08
0.17	1.1E-07	4.4E-08	2.9E-08	1.7E-07	1.7E-07	2.4E-08	9.4E-08	1.4E-07	1.3E-07
0.18	1.6E-07	5.9E-08	4.0E-08	2.4E-07	2.4E-07	3.2E-08	1.2E-07	1.8E-07	1.7E-07
0.19	2.3E-07	8.0E-08	5.4E-08	3.5E-07	3.5E-07	4.3E-08	1.6E-07	2.4E-07	2.2E-07
0.2	3.3E-07	1.1E-07	7.2E-08	5.0E-07	4.9E-07	5.6E-08	2.1E-07	3.1E-07	2.9E-07
0.21	4.6E-07	1.4E-07	9.7E-08	7.0E-07	6.9E-07	7.3E-08	2.6E-07	4.0E-07	3.8E-07
0.22	6.4E-07	1.9E-07	1.3E-07	9.9E-07	9.6E-07	9.6E-08	3.4E-07	5.2E-07	4.9E-07
0.23	8.9E-07	2.5E-07	1.7E-07	1.4E-06	1.3E-06	1.2E-07	4.3E-07	6.6E-07	6.3E-07
0.24	1.2E-06	3.2E-07	2.3E-07	1.9E-06	1.8E-06	1.6E-07	5.5E-07	8.5E-07	8.1E-07
0.25	1.7E-06	4.2E-07	3.0E-07	2.7E-06	2.5E-06	2.1E-07	6.9E-07	1.1E-06	1.0E-06
0.26	2.3E-06	5.5E-07	3.9E-07	3.7E-06	3.4E-06	2.6E-07	8.7E-07	1.4E-06	1.3E-06
0.27	3.1E-06	7.1E-07	5.0E-07	5.1E-06	4.7E-06	3.4E-07	1.1E-06	1.7E-06	1.7E-06
0.28	4.2E-06	9.2E-07	6.5E-07	6.9E-06	6.3E-06	4.3E-07	1.4E-06	2.2E-06	2.1E-06
0.29	5.7E-06	1.2E-06	8.5E-07	9.4E-06	8.5E-06	5.4E-07	1.7E-06	2.8E-06	2.6E-06
0.3	7.6E-06	1.5E-06	1.1E-06	1.3E-05	1.1E-05	6.8E-07	2.1E-06	3.5E-06	3.3E-06

APPENDIX F. EXAMPLE CALCULATIONS

Temperature Profile

For operating conditions 35 %wb and 15 hz,

$$\mathrm{T_{slope}} = \frac{\mathrm{T_{final}} - T_{intial}}{L_{total}} \frac{83.5\ °\mathrm{C} - 23.2\ °\mathrm{C}}{0.3\ \mathrm{m}} = 201\ \frac{°\mathrm{C}}{\mathrm{m}} = 201 \frac{K}{m}$$

$$T_{intercept} = 23.2°C$$

Rate Constant, k

From Figure 5.2

$$\ln(k) = -15511 * \frac{1}{T} + 26.466$$

Where T is in Kelvin. Thus, at 83.5°C

$$\ln(k) = -15511 * \frac{1}{83.5\ °C + 273.2} + 26.466$$

$$\boxed{k = 4.1\ \cdot 10^{-08}\ 1/s}$$

Average Temperature

For operating conditions, 35 %wb and 15 hz motor speed

$$\Delta\mathrm{T} = \frac{\mathrm{T_{final}} - \mathrm{T_{intial}}}{2} = \frac{83.5\ °\mathrm{C} - 23.2\ °\mathrm{C}}{2} = 53.4\ °\mathrm{C}$$

Motor Speed to Screw Speed

At a motor speed of 50 hz,

$$\text{N (rpm)} = \text{ Motor Speed (hz)} * 60 * \text{r}$$

where r is the reduction ratio going from motor speed to screw speed and is equal to the maximum screw speed/maximum motor speed

$$\text{N} = 50 * 60 * 0.033$$

$$\boxed{N = 100\, rpm}$$

Newtonian Shear Rate

At 35 %wb, 15 hz

$$Channel\ \dot{\gamma} = \frac{\pi \cdot D \cdot N}{60 \cdot H} = \boxed{15.2\ 1/s}$$

$$Clearance\ \dot{\gamma}_{\delta} = \frac{\pi \cdot D \cdot N}{60 \cdot \delta} = \boxed{27.3 \frac{1}{s}}$$

$$Die\ \ \dot{\gamma}_{die} = \frac{3n+1}{4n} \cdot \frac{4 \cdot F}{\pi \cdot \rho \cdot R^3} = \boxed{33.97 \frac{1}{s}}$$

$$Steam\ Lock\ \ \dot{\gamma}_{sl} = \frac{\pi N sl}{sl_{gap}} = \boxed{8.88 \frac{1}{s}}$$

Using Penner (2011) ratios for an average Newtonian shear rate,

$$\dot{\gamma}_{Newtonian} = Channel\ \dot{\gamma} * 0.897 + 0.103 * Clearance\ \dot{\gamma}_{\delta}$$

$$\boxed{\dot{\gamma}_{Newtonian} = 16.5\frac{1}{s}}$$

Newtonian Viscosity

$$Channel\ \eta = K\left(\frac{\pi \cdot D \cdot N}{60 \cdot H}\right)^{n-1} = \boxed{2.15 \cdot 10^4\ Pa \cdot s^n}$$

$$Clearance\ \eta = K\left(\frac{\pi \cdot D \cdot N}{60 \cdot \delta}\right)^{n-1} = \boxed{1.43 \cdot 10^4\ Pa \cdot s^n}$$

$$Die\ \ \eta = K\left(\frac{3n+1}{4n} \cdot \frac{4 \cdot F}{\pi \cdot \rho \cdot R^3}\right)^{n-1} = \boxed{1.23 \cdot 10^4\ Pa \cdot s^n}$$

$$Steam\ Lock\ \ \eta = K\left(\frac{\pi N sl}{sl_{gap}}\right)^{n-1} = \boxed{3.13 \cdot 10^4\ Pa \cdot s^n}$$

Note that the viscosity used in the Harper Equation was determined using Ponrajan's master curve equation for viscosity inside the extruder as outlined in Chapter 5.

Harper Equation for Total Energy

At 35 %wb, 15 hz

$$E(\dot{\gamma}) = p \cdot \frac{(\pi DN)^2 L}{sin\theta}\left[\eta \cdot \frac{W}{H}(\cos\theta^2 + 4\sin\theta^2) + \eta_\delta \cdot \frac{e}{\delta} + \cdot \eta_{sl} \cdot \frac{sl}{sl_{gap}}\right] + p \cdot \frac{\pi DNWH}{2} \cdot \Delta P_{backmix}\cos\theta + \dot{\gamma}_{die} \cdot \eta_d \cdot Q$$

Where Q in the Harper Equation is in Kg/s

$$E(\dot{\gamma}) = 8051.9\ Watts$$

Dividing by 1000 J/kW and by the average flow rate in kg/hr,

$$\frac{E(\dot{\gamma})}{1000 * Q(\frac{kg}{hr})} = \frac{8051.9\ Watts}{1000 * 25.0} = 0.32\ kW * hr/kg$$

Where,

$$\Delta P_{backmix} = \frac{G1 \cdot Fdt}{\frac{K}{\eta_d} + \frac{G2 \cdot Fdt}{\eta \cdot L}}$$

And the values of G1, G2, and K can be calculated as follows:

$$G1 = \pi^2 D^2 H \left(1 - \frac{e \cdot p}{\pi \cdot D \cdot \sin\theta}\right) \sin\theta \cos\theta$$

$$G2 = \frac{\pi}{12} \cdot D \cdot H^3 \left(1 - \frac{e \cdot p}{\pi \cdot D \cdot \sin\theta}\right) \sin\theta^2$$

$$K = \frac{\pi \left(\frac{D_{die}}{2}\right)^2}{8 \cdot L_{die}}$$

Specific Energy Consumption

Using solver, the shear rate can be determined using the Equation for SEC

Sensible Heat Energy

At 35 %wb, 15 hz, Q in kW can be determined as follows:

$$\mathrm{Q} = \frac{\dot{\mathrm{m}}\mathrm{C}\Delta\mathrm{T}}{1000 * 3600}$$

$$Q = 25.0 \frac{kg}{hr} * 9.05 * 10^3 \frac{J}{kg * K} 60.3 = \boxed{3.78\ kW}$$

Shear Stress

At 35 %wb, 15 hz, shear stress in kPa can be determined as follows:

$$\tau = \frac{\eta(\dot{\gamma}) * \dot{\gamma}}{1000} = 376.7\ kPa$$

Note that this is the effective shear stress (i.e. using the effective shear rate and viscosity determined in section 5.3).

Extruder Dimensions

Variable	Description	Value	Unit
D	Internal barrel diameter, accounting for the clearance	0.040007	m
δ	Clearance between the barrel and the screw	0.0023	m
e	Flight top width	0.00145	m
F	Volumetric Flow Rate	-	m^3/s
F_p	Figure 2 from Harper (1989)	*0.68*	-
F_d	Figure 2 from Harper (1989)	*0.70*	-
tan θ		0.132	-
F_{pe}	Figure 7 from Harper (1989) (L/D = 11, $\theta = 7.5$)	1.04	-
F_{de}	Figure 6 from Harper (1989) (tan θ= 0.132)	1.0	-
F_{dc}	Figure 8 from Harper (1989) (H/D =0.101, $\theta = 7.5$)	1.04	-
F_{pc}	Figure 9 from Harper (1989) (H/D =0.101, $\theta = 7.5$)	1.1	-
Fdt	Fd*Fde*Fdc	0.73	-
G1	Corrected Flow Equation (Penner, 2011)	7.70E-06	m^3
G2	Corrected Flow Equation (Penner, 2011)	1.1459E-08	m^3
H	Channel depth (Penner, 2011)	0.00413	m
K	(Penner, 2011)	1.44E+05	Pa*s
k	Rate constant	*See "Rate Constant, k" calculation*	1/s
L	Lscrew + Lsl	0.06037	m
L_{total}	Length of extruder, measured	0.3	m
L_{screw}	Length of 1 screw section plus 5 washers (Penner, 2011)	0.053527	m
L_{sl}	Steam lock length for one steam lock (Penner, 2011)	0.00807	m
$\dot{m}$	Mass flow rate	-	Kg/hr
N	Screw speed	*See screw speed calculation*	*rpm*

n	Flow behavior index	0.3028	
p	Single helical flight	1	single helical flight
Q	Sensible Heat	*See Sensible Heat Calculation*	kW
R	D/2	0.0066375	mm
r	Reduction ratio	0.033	
sl	Width of steam lock (Penner, 2011)	0.0080772	m
sl_{gap}	Gap between outermost diameter and the barrel, material must pass through here before exiting this screw section (Penner, 2011)	0.00142875	m
T	Temperature		Kelvin/°C
θ	Helix angel at flight top (Penner, 2011)	0.13089958	radians
W	Channel width perpendicular to flight (Penner, 2011)	0.00692	m