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Selectivity of Infrared Heat Treatment on Inactivation of Mycotoxigenic Fungi on Stored Grain

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Selectivity of Infrared Heat Treatment on Inactivation of Mycotoxigenic Fungi on Stored Grain

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by

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Abstract

Selective Infrared (IR) heating holds great potential to decontaminate spores of unsafe fungi in corn. The objectives for this study were to investigate the impact of exposing corn to infrared energy at selected peak wavelengths (λ), infrared intensities and treatment durations, followed by tempering for further inactivation of microbes on the grain and explore a method for decontaminating *Aspergillus flavus (A. flavus)* spores on corn. Freshly harvested corn with initial moisture contents (IMCs) of 16%, 20%, and 24% wet basis (w.b.) were used. The corn samples were treated at different infrared wavelengths (3.2, 4.5, and 5.8 μm) for 20, 40 and 60 s at product-to-emitter gap sizes (PEG) of 110, 275, 440 mm. This was then followed by tempering the grain at 70^{o}C for 4 hrs. Fungal analyses were assessed in terms of colony forming units per gram of treated corn (CFU/g). Internal transcribed spacer (ITS) amplicon sequencing techniques were also used to identify and quantify the magnitudes of surviving fungi following treatments. The mean of the mold count of control samples were 5.95 ± 0.1 Log (CFU/g). Samples treated at wavelength 3.2 μm, PEG of 110 mm (intensity of 15.71 kW/m^2) and heating duration of 60 s resulted in the highest microbial load reduction of 3.0, 4.7, 4.9 Log CFU/g of grain for MC 16%, 20%, and 24% (w.b.), respectively. Tempering treatment further reduced the microbial load at each infrared treatment condition. *Aspergillus* genus was the most abundant mycotoxin producing fungi on the non-tempered corn samples while *Penicllium* was the most abundant on the tempered samples compared to the population of other fungi. After samples were inoculated with *A. flavus*, treatments at wavelength of 3.2 μm, product-to-emitter-gap sizes (PEG) of 110 mm and corn MC of 24% wet basis (w.b.) resulted in the greatest *A. flavus* load reduction of 4 Log CFU/g for non-tempered and tempered samples. This work showed that decontamination of

harmful fungi, known to exist on corn, may be enhanced by infrared treatments at selected wavelengths.

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Chapter 1: Introduction

Corn is the main grain used in the production of animal feed in the United States (USDA, 2018). A small portion of corn is processed for use as corn cereal, corn starch, corn oil and corn syrup for human consumption, but it is primarily used for livestock feed, and manufactured goods such as pet foods (USDA, 2018). The United States is a significant player in the world corn trade market, exporting to other nations between 10% and 20% of its corn crop (USDA, 2018).

One of the most important economic considerations in corn processing is preventing the growth of fungi on the kernels. Fungi are associated with an enormous diversity of habitats which include grains. Under some conditions, some fungal species may produce secondary metabolites known as mycotoxins. Mycotoxins once created are highly stable and heat-resistant compounds that readily survive the temperatures of food processing and if consumed poses serious health risk to humans and animals (Peraica et al., 1999; Bullerman et al, 2007).

Prevention of proliferation of these aflatoxigenic fungi on food is the most desirable and effective known method for controlling aflatoxin production. Various strategies are used to control the risk of aflatoxin production before crop harvest, including selective breeding to advance the development of varieties resistant to the toxin-producing fungi, proper cultural practices, such as choice of planting and harvest dates, tillage practices, crop rotation, plant population, irrigation, and sanitation, and applying crop protection chemicals or biological controls to mitigate aflatoxin contamination (Atungulu, 2015a, b). During post-harvest period, the primary method to discourage aflatoxin formation on grains is though implementation of proper drying and storage practices; the goal is to discourage development of the aflatoxigenic fungal species - *Aspergillus flavus* and *Aspergillus parasiticus* (Atungulu et al., 2015a, b). The

conidia (spores) of these fungal species are prevalent in the air and therefore are common contaminant of grains with corn frequently victimized. The spores of aflatoxigenic fungi are very heat tolerant, and have been known to survive conventional convective hot-air drying temperatures for grains (Wilson et al., 2015). This, therefore increases the risk of toxin production and potential contamination when dried grain is mixed with other moist material in processing lines. Therefore, any innovation regarding corn drying practices must be able to produce a product that is, if not, equal to or better than the present quality of corn. As an alternative to conventional corn drying methods, Selective infrared (IR) heating technology was explored for mycotoxigenic fungi inactivation.

Hypothesis

Infrared (IR) energy supplied by broadband wavelength could inactivate microbes but not completely. Understanding the interactions between the applied wavelength and fungi response is important. Hence, within the IR band some selected wavelengths may have unique interactions with mycotoxigenic fungi resulting in further inactivation.

Objectives

1) Investigate the effect of IR heating and tempering at varied selected peak wavelengths (λ), infrared intensities and treatment durations on inactivation of fungi generally prevalent on corn.
2) Investigate the effects of infrared wavelengths, treatment durations, and intensity on inactivation of mycotoxigenic fungi, specifically of Aspergillus genus.

3) Investigate the suitable combinations of IR heating duration, intensity, wavelengths, and moisture content (MC), followed by tempering treatments to maximize decontamination of A. flavus mold spores.

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Chapter 2: Literature Review

Infrared

Fundamental principles of infrared radiation

In 1782 William Herschel discovered the presence of invisible heating rays, now known as infrared (IR) radiation (Ring, 2000; Rowan-Robinson, 2013). Any object which has a temperature above absolute zero (that is °K or - 273 °C) emits IR radiation in the form of electromagnetic waves and is produced by the acceleration of electric charges. Electromagnetic waves can be imagined as self-propagating transverse oscillating wave of electric and magnetic fields.

Infrared radiation is a band in the electromagnetic spectrum with wavelength less than that of microwave but greater than that of visible light (Pan and Atungulu, 2010) (Figure 1). The IR radiation band is often considered to comprise wavelengths from about 0.75 μm to about 1000 μm (Pan and Atungulu, 2010). The International Commission on Illumination (CIE) recommends that IR radiation be divided into three general regions: Near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). NIR, MIR, and FIR spectral ranges are 0.75 to 1.4, 1.4 to 3, and 3 to 1000 μm, respectively (Sakai and Hanzawa, 1994; Pan and Atungulu, 2010; Wilson et al., 2015; Okeyo, 2016). The CIE classification is widely reported and adopted in food processing.

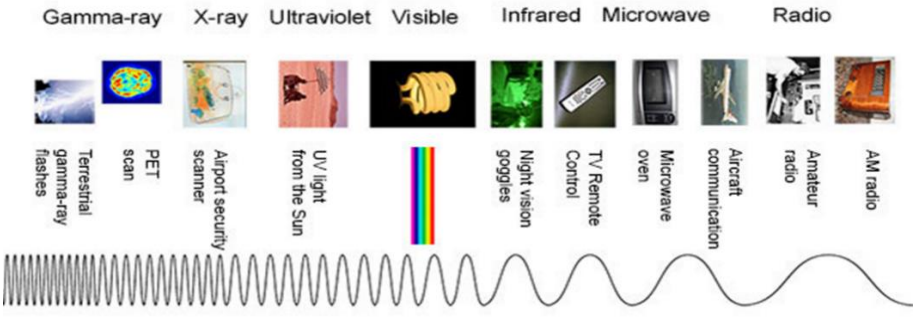

Figure 1. Electromagnetic Spectrum

Infrared radiation incident on a product can be reflected, absorbed or transmitted. For a molecule to display IR absorptions, the electrical dipole moment of the molecule must change during movement (Stuart, 2004). A blackbody is an ideal body, which absorbs all incident radiation, and also emits the maximum radiant energy (Pan and Atungulu, 2010). As an ideal body, it is compared to a real body to describe the ability of a real body to emit and absorb thermal radiation. The emissivity of a surface is the ratio of the energy flux emitted by the real body to the flux emitted by a blackbody at the same temperature (Pan and Atungulu, 2010).

Basic laws of infrared radiation

There are three basic laws for electromagnetic radiation that describe the type and quantity of energy emitted by an object. The laws are Stefan-Boltzman's law, Planck's law, and Wien's law on displacement (Sakai and Hanzawa, 1994; Dagerskog and Österström, 1979; Krishnamurth et al., 2008). The laws are summarized in table 1.

Table 1. Basic Laws of Infrared Radiation

Laws	Explanation
Stefan–Boltzmann's law $E_b(T) = n^2\sigma T^4$	Gives the total power radiated ($E_b(T)$) at a specific temperature from an infrared source σ: Stefan–Boltzmann constant (5.669×10^{-8} W/m^{2}K^4) n: refractive index of the medium
Planck's law $E_{b\lambda} = \frac{2\pi hc_0^2}{n^2\lambda^5[e^{\frac{hc_0}{n\lambda kT}} - 1]}$	Gives spectral blackbody emissive power distribution $E_{b\lambda}(T, \lambda)$ k: Boltzmann's constant (1.3806×10^{-23} J/K), n: refractive index of the medium (n for vacuum is 1 and, for most gases, n is very close to unity) λ: the wavelength (μm) T: source temperature (K) c_0: speed of light (km/s) h: Planck's constant (6.626×10^{-34} J·s)
Wien's displacement law $\lambda_{max} = \frac{2898}{T}$	Gives the peak wavelength (λ_{max}), where spectral distribution of radiation emitted by a blackbody reaches maximum emissive power T: emitting body temperature (K)

The amount of IR radiation that occurs on any surface is spectrally dependent. The energy from an emitter consists of different wavelengths and the fraction of the radiation in each band depends on the temperature and emissivity of the emitter. The wavelength at which the maximum radiation happens is dictated by the IR heating element temperature (Figure 2).

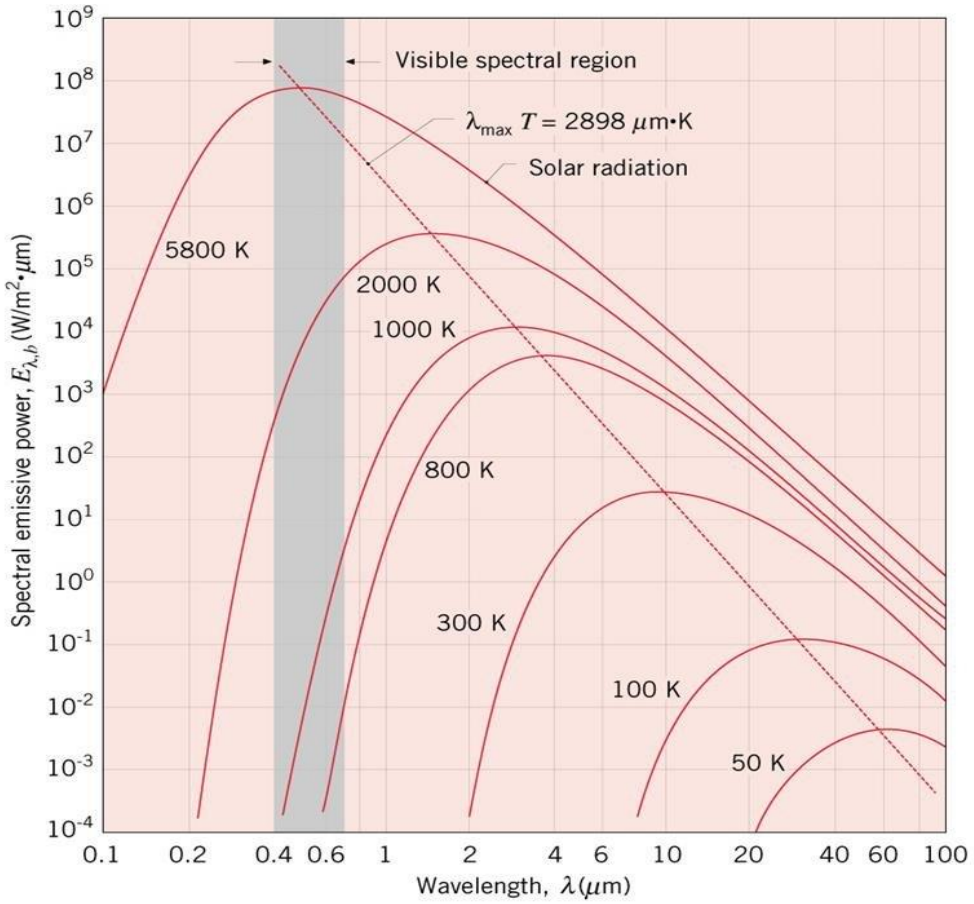

Figure 2. Spectral Peak Distribution

Interaction of infrared radiation with food materials

Infrared energy does not depend on air for transmission and is converted to heat upon absorption by the material being heated. Because air and gases absorb very little IR energy, the IR heating process provides for efficient heat transfer without contact between the heat source and the material being heated.

Foodstuffs which contains mostly water absorb MIR and FIR energy most efficiently through various modes of vibrations, which leads to the radiative heating process. Vibrations may involve changing the length of the bond (stretching) or the angle of the bond (bending). Some bonds may be either symmetric or asymmetric (Stuart, 2004). When used to heat or dry moist materials, IR radiation impinges on the exposed material and penetrates it, and then the

radiation energy is converted into heat (Ginzburg, 1969). Infrared radiation can penetrate significantly into the food product, and such penetration may have dramatic effect on surface moisture and surface temperature (table 2).

Table 2. Penetration Depth of Infrared Energy into Food Products (Ginzburg, 1969)

Product	Spectral peak, μm	Penetration depth, mm
Dough, Wheat	1.0	4-6
Bread, Wheat	1.0	11-12
Biscuit	1.0	4
	0.88	12
Grain, wheat	1.0	2
Carrot	1.0	1.5
Tomato Paste, 70-85% Moisture Content	1.0	1
Potatoes, Raw	1.0	6
Potatoes, Dry	0.88	15-18
Apples, Raw	1.16	4.1
	1.65	5.9

Infrared radiation applications in food industry

Infrared treatments have been associated with merits of higher energy transfer rate, shorter drying duration, lesser environmental footprints, and better or comparable product quality compared to convective heated air treatments (Ratti and Mujumdar, 1995; Wang et al., 2014).

Infrared heating has been developed in various sectors of the food industry and is often used in processes such as drying, frying, baking, blanching and roasting (Nasiroglu and Kocabiyik, 2009; Zhu and Pan, 2009; Aghajanzadeh et al., 2016; Bagheri et al., 2016; Salehi et al., 2016; Mohammadi et al., 2018). Infrared heating is also used to heat and cook soybeans, cereals, cacao beans and nuts, and ready-to-eat products (Ratti and Mujumdar, 1995; Nowak and Piotr, 2004).

Infrared radiation has also been used in the food industry to inactivate pathogenic microbes in liquid and solid foods (Khlangwiset et al., 2011). The effectiveness of IR heating on microbial inactivation depends on the following parameters: administered IR power level, surface temperature of food products, peak wavelength, bandwidth of IR heating source, product thickness, types of microorganisms, moisture content of product, and chemical matrix of food material (Khlangwiset et al., 2011).

Concept of selective infrared heating

The spectral distribution peak at a specified wavelength is defined by Wein's law (Siegel and Howell, 2002). For most food, the optimum IR dehydration process uses wavelengths below 4.2 μm (Lentz et al., 1995). The importance of IR emitting wavelength for the thermal processing of dough was studied by Lentz et al. (1995). When the IR spectral emission was not consistent with the wavelengths best absorbed by the dough (approximately 0.8 – 1.3 μm), poor heating of the surface and interior was observed. Jun (2002) developed a new selective FIR heating system that demonstrated the importance of optical properties when electromagnetic radiation is used for food processing. The system had the capability to selectively heat higher absorbing components to a greater extent using optical band pass filters that can emit radiation in the spectral ranges as

needed. Applicability of this technique was demonstrated by selective heating of soy protein and glucose.

Generally, IR heating has been reported to improve fungal inactivation (Wilson et al, 2017). Such effort may be extended for prevention of mycotoxin contamination of grains. Specifically, the use of selective IR heating of harmful mycotoxigenic molds has not been reported. There seem to be a new avenue to employ such approach to target mycotoxigenic fungi and improve the efficacy of the inactivation process.

Mycotoxigenic fungi

Types of mycotoxigenic fungi

Fungi that produces mycotoxin are referred to as mycotoxigenic fungi. The main mycotoxigenic fungi are *Aspergillus*, *Fusarium*, and *Penicillium* (Sadhasivam et al., 2017). At the moment *Aspergillus* genus comprises 339 known species (Perrone and Gallo, 2017). The genus *Fusarium* comprises around 70 well-known species, identified by using a polyphasic approach (Munkvold, 2017). *Fusarium* is one of the most economically important fungal genera because of its contribution to agricultural commodity yield loss due to plant pathogenic activity (Munkvold, 2017). *Penicillium* are very diverse and cosmopolite fungi; about 350 species are recognized within this genus (Perrone and Susca, 2017).

All Aspergillus and Penicillium species are either growing in crops without obvious signs of pathogenicity, or invading crops after harvest and produce toxins during drying and storage. In contrast, the important Fusarium species infect crops before harvest. There are two types of mycotoxigenic fungi: field (plant pathogens) and storage (saprophytic) fungi (Sadhasivam et al., 2017). The field fungi invade and produce their toxins before harvesting while the storage fungi

become a problem after harvesting. Environmental stressors such as water activity and temperature can affect growth, the expression of biosynthetic regulatory genes and production of mycotoxigenic fungi.

Issues associated with mycotoxigenic fungi

Contamination of staple crops by mycotoxin has been a serious issue for human and animal health (Reddy et al., 2008). When fungi colonizes grains, seeds, and feed on the field or in storage there is a possibility of contamination with mycotoxins. Mycotoxins are toxic secondary metabolites produced by filamentous fungi. The Food and Agricultural Organization (FAO) of the United Nations estimated that each year approximately 25% of crops in the world are adulterated by mycotoxins, which cause annual losses of around one billion metric tons of food products (Smith et al., 2016). The most relevant mycotoxins for food safety include aflatoxins, ochatoxin A, fumonisins, zearalenone, and trichothecenes (Reddy et al., 2008) (Figure 3).

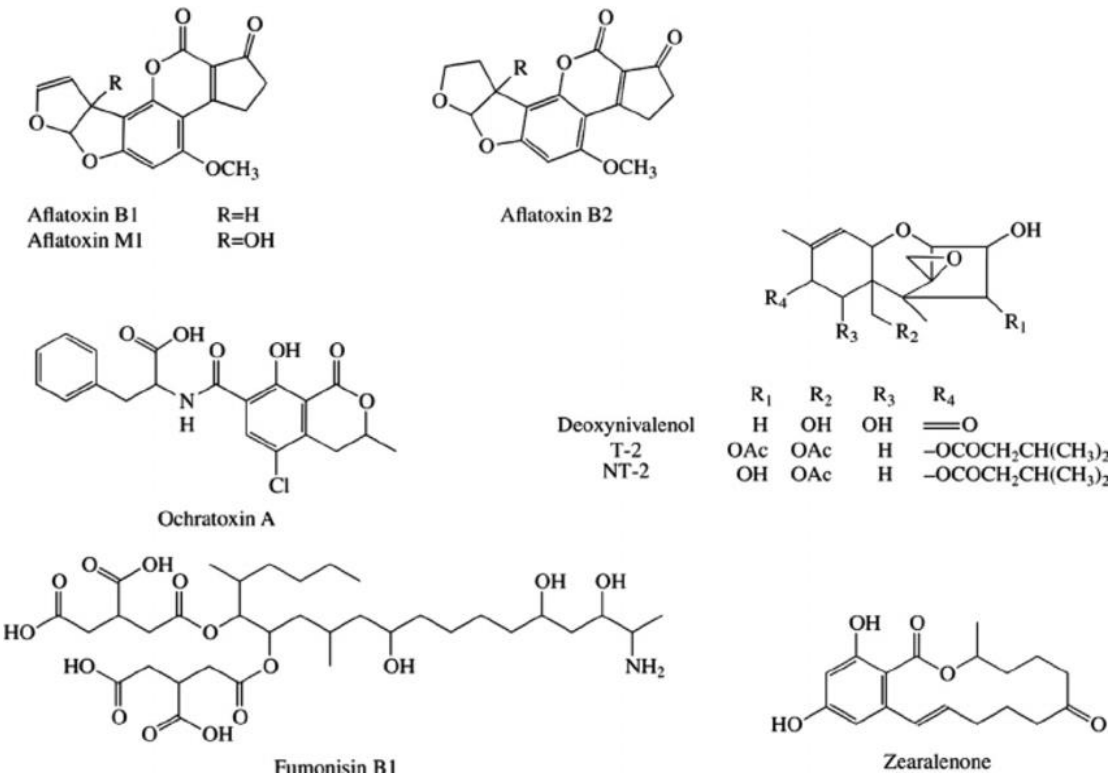

Figure 3. Chemical Structure of most relevant mycotoxins

Mycotoxins are typically hidden contamination aspects that remain far beyond the life cycle of the fungus. Mycotoxins once formed are extremely stable and heat-resistant and can survive the temperatures of food processing (Bullerman et al., 2007). Some mycotoxins, even at low levels, can cause impairment of the immune system and reduce resistance to infections in individuals. Mycotoxins can cause tumors and chronic diseases in vital organs in acute situations or cause high morbidity and premature death in humans and animals (Peraica et al., 1999). Of the mycotoxins, aflatoxins have received a lot of attention than any other due to potent carcinogenic and acute toxicological effects in humans such as liver cancer, growth impairment, and emesis (Williams et al., 2004; Khlangwiset et al., 2011).

Aflatoxins are mainly produced by *Aspergillus flavus* (A. *flavus*) and *Aspergillus parasiticus* (Iqbal et al., 2006; Varga et al., 2011; Pitt et al., 2013). Aflatoxin B1 (AFB1) is the most toxic and carcinogenic of the aflatoxins group; it is mutagenic, immunosuppressive, and

teratogenic (Williams et al., 2004; Bbosa et al., 2013; Villers, P., 2014). AFB1 is produced only by A. *flavus* (Magan et al., 2004; Reddy et al., 2005). Aflatoxin regulatory levels in food and animal feed are issued in several countries. The acceptable limit based on European Union is between 2-12 ppb for B1 and 4-15 ppb for total aflatoxins. A limit of 20 ppb is set for total aflatoxins in all food by United States ([FAO] Food and Agriculture Organization of the United Nations, 2004); Atungulu et al. 2017). Table 3 shows the action levels that should not be exceeded for aflatoxin in human food and animal feed (Atungulu et al., 2017).

Table 3. Food and Drug Administration Action Levels for Aflatoxin in Human Food and Animal Feed

Commodity	Intended Use	Aflatoxin Level (ppb)
Corn and peanut products	finishing (i.e., feedlot) beef cattle	300
Cottonseed meal	beef, cattle, swine, or poultry (regardless of age or breeding status	300
Corn and peanut products	finishing swine of 100 pounds or greater	200
Corn and peanut products	breeding beef cattle, breeding swine, or mature poultry	100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal,	immature animals	20
Corn, peanut products, cottonseed meal, and other animal feed ingredients	dairy animals, for animal species or uses not specified above, or when the intended use is not known	20
Brazil nuts, Foods, Peanuts and Peanut products, Pistachio nuts	Human Consumption	20
Milk	Human Consumption	0.5 (aflatoxin M1)

Fungal detection methods

Traditional methods for evaluating fungal diversity mainly depend on the dilution-plating technique with the use of selective media to identify macroscopic appearance, such as color, rapidity of growth, and diffusible pigment in the agar (Lima and Borba, 2001; Alsohaili and Bani-Hasan, 2018). Also, traditionally microscopy is used to identify hyphal morphology (Gaddeyya et al., 2012). The traditional method tend to overlook fungi that is in mycelial state or takes a long time to grow in culture media. Moreover, most of these methods lead to the isolation of only the most common fungi (Davet and Rouxel, 1997). Molecular techniques can be used for species identification if microscopic morphology does not allow definitive identification (Byrne, 2007).

Molecular identification is quick, sufficient, reproducible and can provide a high degree of specificity to distinguish between the species of fungi, contrary to morphological and biochemical tests used in laboratory diagnosis of fungi (Liu et al., 2000). Molecular identification techniques based on the total extraction of fungal DNA then sequencing polymerase chain reaction (PCR) amplified parts of 18S rRNA genes with fungal primers provide a unique barcode for the identification of different fungal isolates at species level (Hensel and Holden, 1996; Landeweert et al., 2003; Monod et al., 2005; Kabak et al., 2006).

Multiplex PCR is also a molecular technique that provides a fast and precise DNA-based tool. It provides simultaneous amplification of species-specific genes and/or structural or regulatory genes involved in mycotoxin biosynthesis. Multiplex PCR have been successfully applied in a variety of foods and feeds to detect mycotoxigenic fungi (Miller, 1995; Martínez-Culebras et al., 2017).

The internal transcribed spacer (ITS) region is used to classify the Aspergillus section (Henry et al., 2000; Kumeda & Asao, 2001; Godet and Munaut, 2010). The ITS region is the most useful for species identification, as it is the most rapidly evolving part of the rRNA cistron (Schoch et al., 2012; Raja et al., 2017). Due to its ease of amplification, widespread use and an adequately large barcode gap, a consortium of mycologists selected the ITS region as the official barcode for fungi (Schoch et al., 2012; Raja et al., 2017). The use of index primers is also a sequencing tool. Individual samples are added to different pairs of index primers, which allow many samples to be mixed together and sequenced at the same time. After sequencing the software can identify these indexes on each read sequence and the machine then knows which sample to associate the read.

Mycotoxigenic fungi prevention and decontamination strategies

Fungi in crops can be prevented pre-harvest and post-harvest. Pre-harvest prevention strategies include choice of resistant varieties, the use of biological and chemical agents and the management of harvest schedules. Some post-harvest strategies include improved drying methods, good storage conditions, irradiation, the use of natural and chemical agents, limiting the physical damage of grains during harvesting, and avoiding insect infestation (Mohapatra et al., 2017; Ukwuru et al., 2018). Grains contaminated with mycotoxin can be removed physically by hand or by photoelectric detectors, but would be labor intensive, time-consuming and expensive (Suttajit, 1989).

In biological control, the use of microbial antagonists either alone or as part of an integrated control strategy to reduce pesticide inputs has emerged as a promising pre-and post-harvest approach to control mycotoxins in crops (Ukwuru et al., 2018). Several organisms have

been tested for their ability to reduce both fungal infection and mycotoxin contamination, including atoxigenic aspergilli, yeasts, bacteria and fungi (Ukwuru et al., 2018). For example, atoxigenic fungal strains are widely used in several parts of the world, including the Mediterranean, to prevent pre-harvest aflatoxin contamination of crops such as peanuts, pistachios, maize and cotton seed (Ukwuru et al., 2018).

The detoxification of foods contaminated with aflatoxin has been an ongoing challenge for the food industry. Chemical treatment should ensure that the detoxification system can alter the toxin to a non-toxic derivative without affecting the raw product (Ukwuru et al., 2018). Many common chemicals have been introduced to test the efficacy of detoxifying aflatoxin, such as acetic acid, ammonia gas, calcium hydroxide and others (Bankole, 1996 ;Kavita and Reddy, 2000).

Another method used to inhibit growth of fungi is use of essential oils through fumigation. Fumigation with two essential oils (oregano and thyme) inhibited the growth of A. *flavus*, A. *niger*, and A. *ochraceus* in stored wheat (Paster et al., 1995). In another study, essential oils from orange (Citrus sinensis) were most effective against A. *niger* and those from mandarin (Citrus reticulata) were most effective against A. *flavus* (Mannaa and Kim, 2017).

Effect of infrared heat treatments on fungi

Moisture in foods play a significant role in absorption of IR radiation thereby leading to a rapid increase in product surface temperature (Hamanaka et al., 2006). Increasing the power of the IR heating source increases temperature and total energy absorbed by microorganisms in a short duration leading to rapid microbial inactivation (Hamanaka et al., 2006; Bingol et al.,

2011). Increasing product thickness reduces temperature increase in the product thus reducing the microbial inactivation efficiency (Hashimoto et al., 1991; Sawai et al., 1997)

Research has examined the use of IR to inactivate surface microorganisms such as in corn flour, grains, cottage cheese, and many other products (Rosenthal et al., 1996; Jun & Irudayaraj, 2003; Hamanaka et al., 2006). Wilson et al. (2017) reported that intermittent drying of freshly harvested corn with different intensities of IR radiation led to a significant reduction in mold load without affecting the quality of the grain. Sterilization of wheat surface was investigated by Hamanaka et al. (2000) who found that IR has the potential to significantly reduce the bacterial counts on wheat. The growth of microorganisms on rice and wheat grain surfaces decreased by increasing radiation intensity (Sorour, 2006). Table 4 shows the impact of IR heating on fungal load reductions in several studies (Jun and Irudayaraj, 2003).

Table 4. Fungal decontamination of food by infrared heating

Pathogen	Food	Temperature/energy	Time	Log reduction ln(log_{10} CFU/mL)	References
Monilia fructigena	Strawberry	Surface temperature approximately 50 °C	10 s	2.5 to 5.2 log (estimated)	Tanaka et al., 2007
Aspergillus niger spores	Corn meal	72 °C	6 min	1.8	Jun and Irudayaraj, 2003
Aspergillus niger spores	Corn meal	68 °C (with an optical filter: 5.45 to 12.23 μm)	6 min	2.3	Jun and Irudayaraj, 2003
*Fusarium proliferatum*spores	Corn meal	72 °C	6 min	1.4	Jun and Irudayaraj, 2003
*Fusarium proliferatum*spores	Corn meal	68 °C (with an optical filter: 5.45 to 12.23 μm)	6 min	1.95	Jun and Irudayaraj, 2003

Maninder et al. (2001) looked at the potential of IR to decontaminate aspergillus on mung bean. They found that treating the sample at an intensity of 0.299 kW/m^2 at 70 ° C for 5 min had approximately 5.3 log10 CFU/g reductions of the fungal spore population without substantially changing the biochemical and physical properties of mung bean. Staack et al. (2008) found that greater than 6 log reduction in fungal spores was observed in paprika powder after being treated with IR. Also, Staack et al. (2008) noted that the higher the water activity the greater the log reduction in paprika powder.

Many studies have looked at the effects of IR heat treatment on the decontamination of fungi but they have not sufficiently explored the concept of selectivity of IR heat treatment on inactivation of mycotoxigenic fungi. The goal of this research is to optimize IR heating and decontamination technology, by using selective IR heating approach as a method to mitigate aflatoxin contamination of grains specifically corn. The research is expected to help improve human and animal health though advancement of a new, improved and commercially viable technology for grain processing.

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Chapter 3: Selectivity of Infrared Wavelengths on Inactivation of Microbes on Shelled Corn

Abstract

Selective infrared (IR) heating was explored as a technique for inactivating microbial load on shelled corn. The impact of exposing corn with different initial moisture contents (MCs) to infrared energy at selected peak wavelengths (λ), infrared intensities and treatment durations, followed by tempering was evaluated. Microbial analyses were assessed in terms of colony forming units per gram of treated corn (CFU/g). Greater mold load reductions were observed at higher heating duration and higher MC. Up to 5 log reduction of microbial load was achieved by treating corn of initial MC of 24% wet basis (w.b.) with IR at wavelength of 3.2 μm (intensity of 15.71 kW/m^2) for only 60 s. Addition of tempering resulted in further 0.2 log reduction of the microbial load. This work showed that the process of inactivating mold spores on corn kernel using infrared could be optimized by targeting the specific wavelengths within the broader infrared electromagnetic spectrum.

Keywords: Infrared heating, selected peak wavelengths, microbial load, infrared intensity.

Introduction

The growing concern for food safety, quality and storage has prompted research into developing cost-efficient approaches for microbial decontamination of grains to reduce crop losses and consumer health hazards (Schmidt et al., 2018a). The grain industry uses both chemical and thermal treatments to achieve microbial decontamination. A few of the chemicals currently used in the industry includes hydrogen peroxide, sodium hypochlorite, acetic acid, sorbate, propionate, and quaternary ammonium compounds (Schmidt et al., 2018a). Examples of thermal treatments used include heated air and radiation (Krishnamurthy et al., 2008; Schmidt et al., 2018a). Some studies have also reported utilization of enzymes for microbial decontamination in the food industry (Klein and Lurie, 1991; Schmidt et al., 2018b). However, utilization of ionizing radiation treatments such as gamma rays or electron beam (e-beam) have been shown to be rapid, efficient, safe and environmentally friendly methods for inactivation of pathogenic and toxic microorganisms (Schmidt et al., 2018b).

Of the thermal radiation treatments, infrared (IR) heating technology has been gaining greater attention for microbial decontamination (Hamanaka et al., 2000; Hamanaka et al., 2006; Wilson et al., 2015). The IR spectrum can be classified into three general regions: Near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). The NIR, MIR, and FIR spectral ranges are 0.75 to 1.4, 1.4 to 3, and 3 to 1000 μm, respectively (Sakai and Hanzawa, 1994; Pan and Atungulu, 2010; Wilson et al., 2015). The amount of energy that is absorbed, reflected or transmitted varies with the wavelength of the IR energy and with different materials and surface conditions (Pan and Atungulu, 2010). Fundamentally, the IR heat transfer is very efficient and does not require contact between the heat source and the material being heated (Pan and Atungulu, 2010). IR treatments have been associated with merits of higher energy transfer rate,

shorter drying duration, smaller environmental footprints, and better or comparable product quality compared to conventional heating techniques (Krishnamurthy et al., 2008). However, IR heating can cause high heat flux on the surface of the heated product, leading to case-hardening (Wilson et al., 2017). Therefore, it is important to incorporate a tempering step to allow the redistribution of moisture in the IR heated material and eliminate development of large moisture gradients within the kernel (Li, et al., 1998; Nishiyama et al., 2006). During the tempering process, the heated material is allowed to rest at a constant temperature for a while under hermetic conditions before the next cycle of heating. IR heating followed by a tempering process may allow further decontamination of mold on the heated material.

Research has examined the use of IR energy supplied by broadband wavelength to decontaminate surface microorganisms on food such as corn flour, grains, cottage cheese, mung bean, paprika and many other products (Rosenthal et al., 1996; Maninder et al., 2001; Jun & Irudayaraj, 2003; Hamanaka et al., 2006; Staack stack et al., 2008; Wilson et al., 2017). Although many studies have observed significant reduction in microbial load after treatment of products with IR energy, most of the reported studies used energy from broadband emitters. The emitters used in those studies supplied the energy from a wide range of wavelengths. It is evident from those studies that complete decontamination of microbes was not achieved. Based on type, chemistry and cellular DNA sensitivity, microbes may respond or interact with IR wavelengths differently. Therefore, the concept of selectivity of IR wavelength on decontamination of microbes needs to be explored exhaustively. Within the IR band some selected wavelengths may have unique interactions with microbes resulting in improved decontamination and overall energy efficiency of the treatment.

The objective of this study was to investigate the impact of exposing corn to infrared energy at selected peak wavelengths, infrared intensities and treatment durations, followed by tempering on inactivation of microbes.

Materials and methods

Corn samples

Freshly-harvested corn at initial MCs of 16%, 20%, and 24% wet basis (w.b.) was procured from the University of Arkansas, Division of Agriculture Experiment Station for use in the experiments. The samples were cleaned by sorting and removing any material other than grain and stored in a laboratory cold room set at 4^{o}C until use. Before conducting any experiments, the samples were retrieved and allowed to equilibrate with room conditions. The MCs of the samples were determined by using an AM 5200 Grain Moisture Tester (PERTEN Instruments, Hagerstown, Sweden). A 100 g of corn was massed out and used for each treatment.

Infrared heating device

The lab-assembled IR dryer is equipped with ceramic infrared (CIR) emitters ((Tempco Electric Heater Corporation, Wood Dale, IL). The CIR emitters contain high temperature insulation blankets in the wiring compartment and is housed in a low profile 20 gauge Aluminized steel or stainless steel box that make the system resistant to thermal cracking. The emitter has a metamorphic yellow color (cold) and changes to orange when hot. The standard operational voltage includes 220 – 240 V with watt density range from 17 to 54.3 kW/m^2; the temperature generated can be as high as 740^{o}C. The construction and schematic of the equipment that was used is shown in Figure 1.

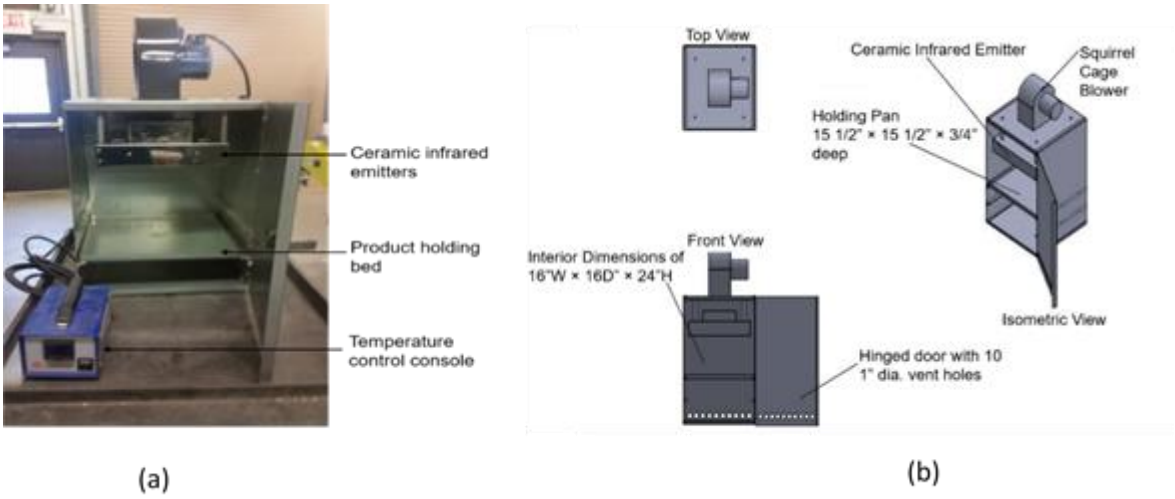

Fig. 1. A laboratory assembled infrared dryer equipped with ceramic infrared emitters (a); Schematic diagram showing different views of the infrared equipment (b).

Measurement of Radiant Energy Transfer

A radiometer (Ophir-Spiricon, LLC, North Logan, UT) was used to determine energy transfer (IR intensity) from IR energy source to the product. The radiometer measures power emitted in kW; the IR intensity was calculated by dividing the supplied power by area of heated black body (equation 1).

$$Energy\ transfer\ (IR\ heating\ intensity) = \frac{Measured\ power\ (kW)}{Heated\ black\ body\ area\ (m^2)} \qquad (1)$$

Infrared treatments

Catalytic IR emitters were used to generate IR energy. The wavelength and total emitted energy was controlled by varying the temperature of IR emitter. Selected peak wavelengths were within the mid and far IR heating region of the electromagnetic spectrum (3.1 μm, 4.5 μm, and 5.8 μm). The IR intensity at the selected product-to-emitter-gap sizes (110, 275, and 440 mm) were determined using a radiometer. The different treatment combinations are shown in table 1. After IR treatments the samples were tempered. In case of tempering treatments, samples were

transferred into glass jars immediately after IR heating, and the jars were sealed air-tight and incubated at the desired tempering temperature of 70°C for 4 hrs.

Table 1. Peak wavelength, product-to-emitter gap size, infrared intensity, heating duration and corn moisture content used in the corn inactivation experiment.

Moisture Content (%w.b.)	Infrared heating duration (s)	Peak wavelength $\lambda_{temp\ (°C)}$ (μm)	Product-to-emitter gap size (mm)	Intensity[£] (kW/m^2)
16	20	~ λ_{226} (5.8)	110	1.55
			275	1.10
			440	0.73
20	40	~ λ_{370} (4.5)	110	4.13
			275	2.87
			440	1.86
24	60	~ λ_{632} (3.2)	110	15.71
			275	10.08
			440	7.27

[£] The intensity (intensity equals measured power (kW) divided by heated black body area (m^2)) was calculated for each wavelength and Product-to-emitter gap size combination.

Due to the large number of experiments to be performed using the original design (Table1), a custom experiment design (DOE) was performed to reduce the experimental load. The purpose of custom DOE is to separate from the many trivial factors that have negligible effects the few important factors that have a significant impact on the response. In a relatively small experiment, custom experiment design allows study of a large number of factors. The custom design creates a computer-generated design to address a broad variety of problems within a unified framework (Design of Experiments, JMP). Continuous, multilevel categorical and mixture factors can be included within the same design (Design of Experiments, JMP). The

custom DOE also allow the specification of hard and very hard-to-change factors for automatic creation of the appropriate designs and has the capability to define factor constraints, model effects and interactions, as well as include center points and/or replicate runs as the design is built (Design of Experiments, JMP).

For this experiment, a custom DOE with response surface model (RSM) was used to obtain a more cost-effective design. A 21 run design was created by the custom design (Table 2). This design was used to generate data and perform analyses reported in the results section.

Table 2. Custom experimental design of different infrared parameters.

Moisture Content (% w.b.)	Peak Wavelength $\lambda_{temp\ (°C)}$ (μm)	Product-to-emitter gap size (mm)	Intensity[£] (kW/m^2)	Heating Duration (s)	Treatments
16	λ_{370} (4.5)	110	4.13	20	1
	λ_{226} (5.8)	110	1.55	60	2
	λ_{632} (3.2)	110	15.71	60	3
	λ_{370} (4.5)	275	2.87	40	4
	λ_{370} (4.5)	440	1.86	60	5
	λ_{632} (3.2)	440	7.27	20	6
	λ_{226} (5.8)	440	0.73	20	7
20	λ_{632} (3.2)	275	10.08	60	8
	λ_{632} (3.2)	275	10.08	40	9
	λ_{370} (4.5)	275	2.87	60	10
	λ_{370} (4.5)	275	2.87	20	11
	λ_{370} (4.5)	440	1.86	40	12
	λ_{370} (4.5)	110	4.13	40	13
	λ_{226} (5.8)	275	1.1	40	14
24	λ_{370} (4.5)	110	4.13	60	15
	λ_{370} (4.5)	440	1.86	20	16
	λ_{632} (3.2)	440	7.27	60	17
	λ_{370} (4.5)	275	2.87	40	18
	λ_{226} (5.8)	110	1.55	20	19
	λ_{226} (5.8)	440	0.73	60	20
	λ_{632} (3.2)	110	15.71	20	21

[£] The intensity (intensity equals measured power (kW) divided by heated black body area (m^2)) was calculated for each wavelength and Product-to-emitter gap size combination.

Mold count determination

Standard procedures for microbial isolation, plating and counting were used (AOAC method 997.02) to determine the corn total mold counts. Phosphate-buffered dilution water (0.5 M, pH = 7.2) were used and sterilized by autoclaving at 121°C (AOAC method 997.02). To determine total mold counts on corn, the samples were masticated using a lab stomacher (Silver Panoramic, iUL, S.A., Barcelona, Spain) to dislodge the microorganisms. A 10 g sample of corn was mixed with 90 mL phosphate-buffered dilution water in a sterile stomacher bag and masticated. The stomacher was set at 240 s and 0.7 stroke/s. This process resulted in corn samples that was pulverized into powder for total microbial load analysis. Serial ten-fold dilutions of the samples were prepared in phosphate-buffered dilution water. The 3M Petrifilm Mold Count Plates (3M Microbiology Product, Minneapolis, MN) were used to enumerate mold counts per manufacturer recommendations. The inoculated plates were stacked to a maximum of 20 units and incubated. Mold Count Plates were incubated at 25°C for 120 h. After incubation, the colony forming units (CFU) on each plate were counted. The appropriate dilution factor, volume, and sample weight were taken into account to obtain the total CFU/g of each sample:

$$T_{cfu} = \frac{P_{cfu}}{D_r} \qquad (2)$$

where, T_{cfu} is total colony forming units per gram of corn (CFU/g), P_{cfu} is colony forming units counted on plate per gram of corn (CFU/g), and D_r is dilution factor (10^{-1} to 10^{-5} times).

Statistical analysis

Analysis of variance (ANOVA) and response surface methodology (RSM) were performed with a statistical software (JMP version 14.0.0, SAS Institute) to determine significant

differences within and among samples. The RSM was used to describe the response-factor relationship. All tests were considered to be significant when $p < 0.05$.

Results and discussion

Implications of selective infrared heat treatment on microbial load

Mold colonies appeared blue, black, yellow, or green in color on the Mold Count Plate. The average mold load on the control samples were 5.95 ± 0.13, 5.85 ± 0.34, and 5.92 ± 0.11 Log (CFU/g) for corn at MC 16, 20 and 24%, respectively. Figure 2 and Figure 3 indicate the contour plots of mold counts in terms of colony forming units (log CFU/g) for IR treatments only. The mean values of the different MC were not significantly different.

Decreasing the PEG resulted in a decrease in mold load on corn kernels (Fig. 2a and Fig. 2b). Figures 2a and 2b also showed that there was higher mold load reduction at a wavelength of 3.2 μm (15.7 kW/m^2, 10.08 kW/m^2, 7.27 kW/m^2) than at a wavelength of 4.5 μm (4.13 kW/m^2, 2.87 kW/m^2, 1.86 kW/m^2) and 5.8 μm (1.55 kW/m^2, 1.10 kW/m^2, 0.73 kW/m^2). Similar results were reported by Sorour, (2006), where microorganism growth on the surfaces of rice and wheat grain decreased with increasing radiation intensity. Also, Hamanaka et al., (2006) and Bingol et al., (2011) reported increasing the power of the IR heating source increases temperature and total energy absorbed by microorganisms in a short duration leading to rapid microbial inactivation.

It was also observed from Figures 2 and 3 that higher heating duration resulted in greater mold load reduction. The tempering step resulted in further reduction of the mold load (Fig. 2b and Fig. 3b) at all of the three wavelengths. Treatments at higher heating durations, lower wavelength and lower PEG were more effective to inactivation of mold.

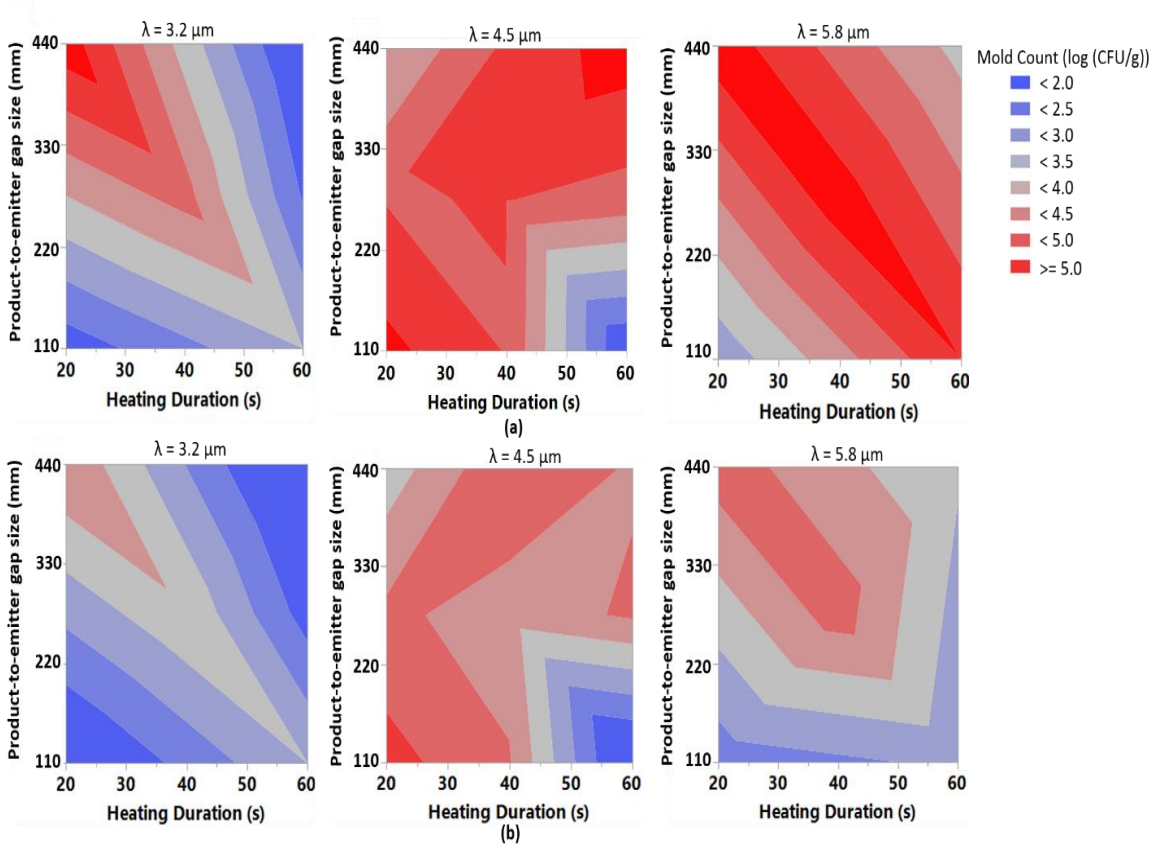

Fig. 2. Contour plots of mold counts (log CFU/g) as influenced by the interaction between heating duration (s), wavelength (µm) and product-to-emitter gap size for infrared heating only (a) and infrared and tempering treatments (b), respectively; CFU means colony forming units; λ signifies wavelength in µm.

The corn kernel MC was an important factor that influenced reduction of the mold load in the samples (Fig. 3). A direct relationship between mold counts and MC was observed; as the MC level increased, the mold counts decreased. The spores on samples with low water activity displayed increased resistance to decontamination (Staack et al., 2008).

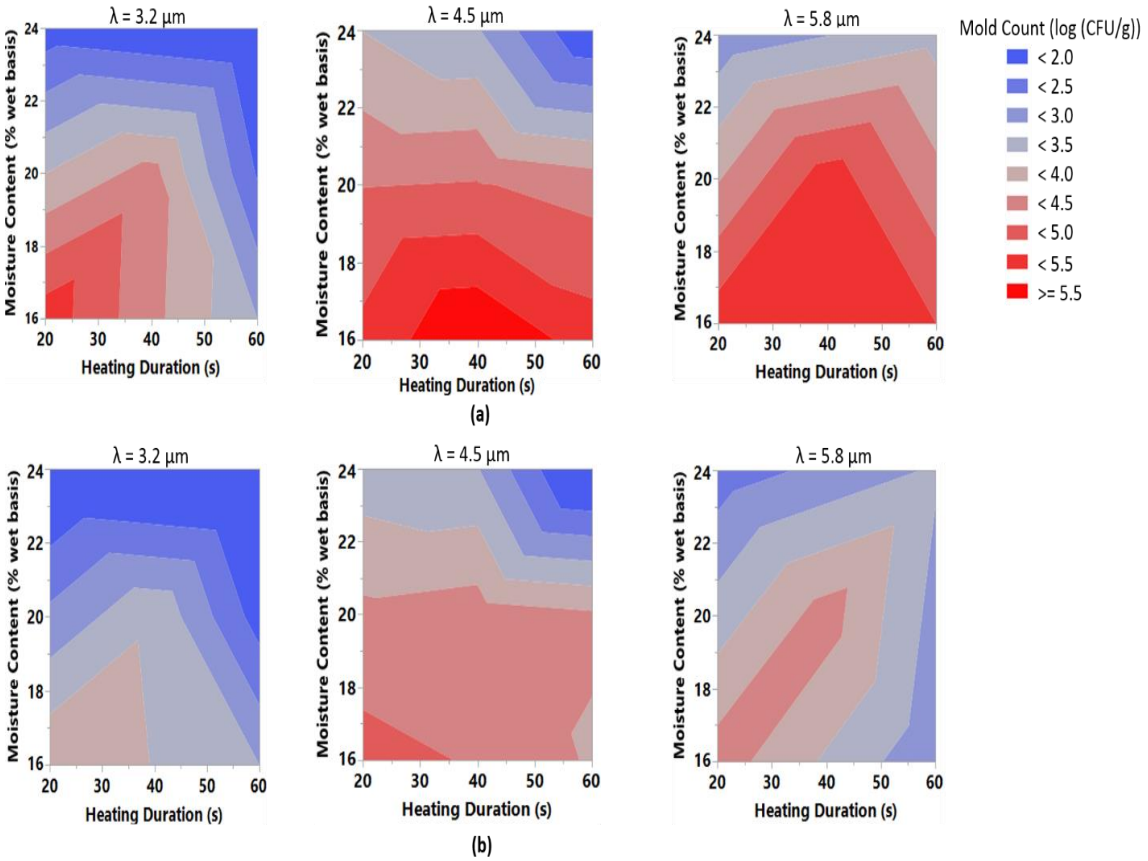

Fig. 3. Contour plots of mold counts (log CFU/g) as influenced by the interaction between heating duration (s), wavelength (µm) and corn moisture content (% w.b.) for infrared heating only (a) and infrared and tempering treatments (b), respectively; CFU means colony forming units; λ signifies wavelength in µm.

Analysis were performed to investigate the correlation between the selected peak wavelength, heating duration, MC, and PEG on the microbial load reduction after IR and tempering treatments. After IR treatment, the effects of selected peak wavelength, heating duration, MC, and PEG had a statistically significant effect on the microbial load response ($p < 0.0001$) (Table 3). The effect of IR treatment combined with tempering also had statistically significant effects on reducing microbial load on the samples (Table 4). The model explained 95% of the variation for IR treatments only and 89% of the variation for IR combined with tempering.

Table 3. Effect test table showing the effects of selective infrared heat treatment on the microbial population of corn.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	39.202018	5.60029	37.4975
Error	13	1.941560	0.14935	Prob >F
Corrected Total	20	41.143578		<0001

Table 4. Effect test table showing the effects of selective infrared heating followed by tempering treatment on the microbial population of corn.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	24.189338	3.45562	15.1240
Error	13	2.970313	0.22849	Prob >F
Corrected Total	20	27.159652		<0001

Table 5 shows the effect summary table for the mold count response. The table list the model effects. Smaller p-values indicate higher significance to the model. The effect summary table for the microbial load response indicates high statistical significance ($p<0.05$) for the main effects (MC, heating duration, product to emitter gap and wavelength). There were also quadratic effects in the model. This means that if the relationship between responses and heating duration were represented by a graph, the optimal responses will not be at the extremes of the experimental region but inside it. There was a statistically significant interaction between the wavelength and the heating duration ($p = 0.0076$) for IR treatments without tempering. This means that wavelength and the heating duration aided in the reduction of the mold count. After infrared and tempering the main effects were still significant, there was quadratic effect between

wavelength and MC. There was also a statistically significant interaction between wavelength and MC (Table 5).

Table 5. Effect summary table showing the effects of moisture content, wavelength, heating duration and product to emitter gap size on the mold count response (Log (CFU/g-grain).

Source	**P Values**	
	Infrared only treatments	**Infrared followed by tempering treatments**
Moisture Content (%w.b.) (16,24)	0.00000	0.00001
Wavelength (μm)(3.2,5.8)	0.00015	0.00891
Heating Duration (s)*Heating Duration (s)	0.00083	> 0.05
Product to Emitter Gap Size (mm)(110,440)	0.00352	0.01755
Heating Duration (s)(20,60)	0.00620	0.00594
Wavelength (μm)*Heating Duration (s)	0.00765	> 0.05
Wavelength (μm)* Wavelength (μm)	0.05057	0.00385
Moisture Content (%w.b.)* Moisture Content (%w.b.)	> 0.05	0.00507
Wavelength (μm)* Moisture Content (%w.b.)	> 0.05	0.04147

Optimization of microbial load reduction after IR and tempering treatments

The factors in this experiment have combinatorial nature because within each factor there are different levels. Therefore, the process must be optimized to determine the best combinations of wavelength, MC, heating duration, and product-to-emitter gap size needed to achieve the greatest responses in terms of lowest mold load. According to the prediction profiles it was determined that minimum levels of microbial load could be obtained at factor settings shown in Figure 4 and Figure 5 for IR treatments only and IR followed by tempering treatments, respectively. Of the possible wavelengths (3.2, 4.5, and 5.8 μm), heating durations (20 s, 40 s, and 60 s), PEGs (110, 275, and 440 mm) and MCs (16, 20, and 24%) it was determined that a wavelength of 3.2 μm, PEG of 110 cm, heating duration of 60 s and MC of 24% w.b. are preferred for optimum inactivation of mold load on corn. The same results were also observed for IR followed by tempering treatments (Fig. 5). It should be noted that a wavelength of 3.2 μm and PEG of 110 mm corresponds to an intensity of 15.71 kW/m^2. At these settings, optimized responses of mold count for IR treatment only and IR followed by tempering treatment should be 0.499 (5.5 log reduction) and 0.229 (5.7 log reduction) Log (CFU/g), respectively. Wilson et al. (2017) reported that increasing the energy (2.39 to 5.55 kW/m^2) of broadband emitters resulted in an average reduction of 2.6 and 2.9 log CFU / g, respectively, at 180 and 30 s of intermittent IR heating. This implies that complete inactivation was not possible with broad band emitters.

In addition to the determination of the optimal factor levels, the prediction profiler also gives insight into the significance of how a factor impact the performance parameter in question. A steep slope indicates that an operational parameter has a significant impact on the given performance parameter, whereas a shallow slope indicates little or no effect on a performance parameter. For mold count response, the operational parameter of MC was determined to be the

most significant for both tempering and tempering combined with IR treatment. This indicates that the effects of increasing the MC contributed the most change to the microbial response.

The last row of plots indicates the desirability trace for each factor (Fig. 4 and Fig. 5). The vertical axis shows both the current desirability and the trace of desirability that result from changing one factor at a time. For IR heat treatment only and IR combined with tempering (Fig. 4 and Fig. 5), a desirability of 0.99 indicates that approximately 99% of the goals to minimize corn mold count were achieved.

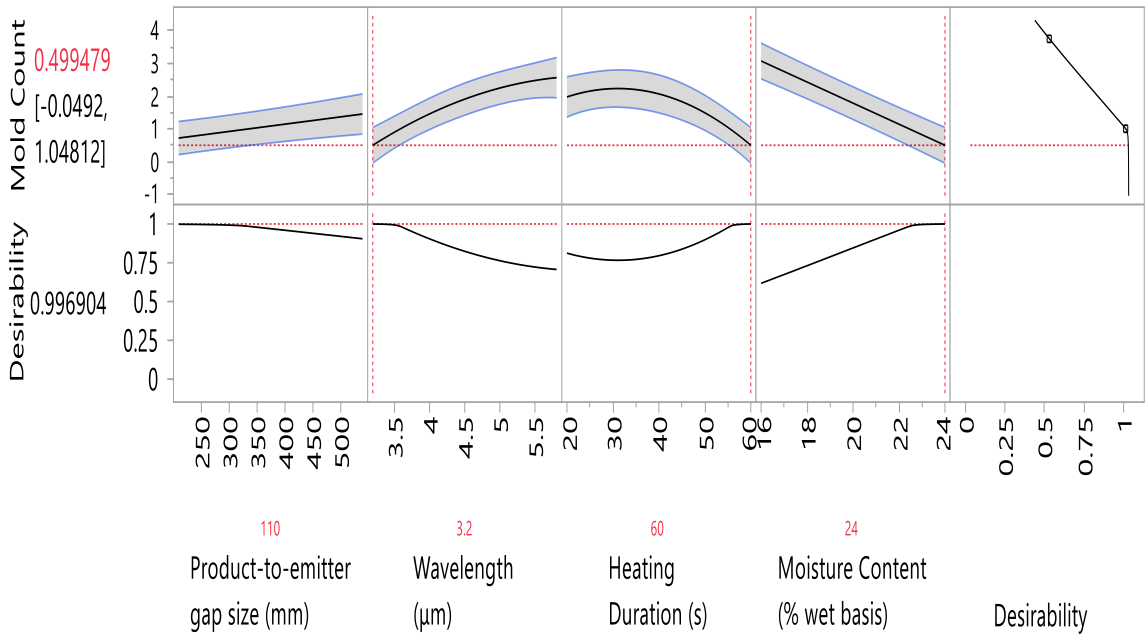

Figure 4: Prediction profile for mold count (Log (CFU/g-grain)) responses with parameter settings product-to-emitter gap size (mm), wavelength (µm), heating duration (s), and corn moisture content (%w.b.) for infrared heat treatments.

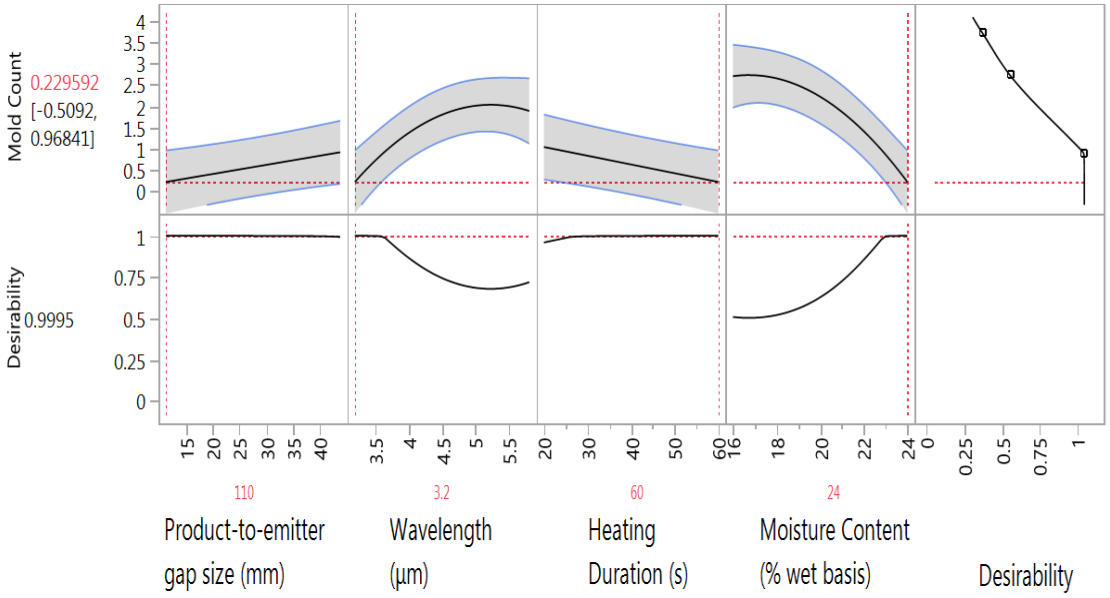

Figure 5: Prediction profile for mold count (Log (CFU/g-grain)) responses with parameter settings product-to-emitter gap size (mm), wavelength (µm), heating duration (s), and corn moisture content (%w.b.) for infrared and tempering treatments.

Conclusion

The results indicate that selective IR treatment approach as opposed to broadband IR treatment could be effective in inactivating molds on shelled corn. It was noted that decreasing the IR peak wavelength and PEG resulted in decreasing microbial load population on corn. An increase in the heating duration also led to a reduction in mold load on the corn samples. Optimization analyses suggest that a wavelength of 3.2 µm (intensity of 15.71 kW/m^2), a heating duration of 60 s, a MC of 24% w.b. and a PEG of 110 mm provide the optimal response in terms of corn mold load reduction. The synergism accorded by tempering allowed for further reduction in microbial load population on corn. This approach might be useful to combat heat tolerant mycotoxin producing molds. The technology if optimized may help reduce corn quantity and quality losses and prevent hazards related to molds.

Acknowledgement

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Chapter 4: Fungal community compositions of shelled corn after selective infrared heat treatments revealed by Illumina high-throughput sequencing

Abstract

Fungi are associated with enormous diversity of habitats which include grains. Once occurring on grains, the spores easily proliferate on high moisture content grains, increasing the risk of toxin production, and potentially contaminate the grain thereby posing important risk to the public. The objective of this study was to characterize effects of using selected peak wavelengths of infrared (IR) heat emission, at various intensities regulated by the product-to-emitter-gap (PEG) (110 mm, 275 mm and 440 mm) and for varied durations, followed by tempering on inactivation responses of fungi that colonize corn kernels. Metagenomics techniques were used to identify and quantify the magnitudes of surviving fungi following treatments at IR peak wavelengths that fall in the mid and far IR heating region of the electromagnetic spectrum (3.2 μm, 4.5 μm and 5.8 μm). Internal transcribed spacer 2 (ITS 2) sequencing was used to characterize the corn microbiome after IR treatments. After IR treatment, Ascomycota was the most abundant phylum for non-tempered and tempered samples. Moisture content (MC) was the most effective variable for reducing the mycotoxigenic fungi of *Aspergillus* and *Penicillin*. The IR heating process shows potential benefits of fungal inactivation which may help the industry prevent potential toxin development on corn.

Keywords: Selective infrared heating, selected peak wavelengths, fungi, product-to-emitter-gap sizes, high-throughput sequencing

Introduction

Fungi growth in food systems is a concern all over the world, not only due to quality and economic losses, but also because of the possibility of mycotoxins synthesis. Mycotoxigenic fungi infecting agricultural crops will produce mycotoxins which are mainly carcinogenic substances (Atungulu and Mohammadi-Shad, 2019). Mycotoxins in grains such as corn pose a severe risk to animal and human health when consumed (Atungulu et al., 2018). Fungi growth and mycotoxins production are more likely in warmer temperature, like U.S. further south (Mohammadi-Shad and Atungulu, 2019). In U.S., high levels of mycotoxins contamination in corn harvested in Mississippi was thought to be related to high temperature conditions (Windham et al., 1999).

The major producers of mycotoxins include species of *Aspergillus*, *Fusarium*, and *Penicillium* (Reddy et al., 2008). Corn grown in United States (U.S.) usually get contaminated with aflatoxins, fumonisins, and deoxynivalenol (DON) (Marasas et al., 1981; Marín et al., 1998; Abbas et al., 2002; Robens and Cardwell, 2003; Wu et al., 2011). Chauhan et al. (2016) conducted a study on fungal infection and aflatoxin contamination in maize collected from Gedeo zone in Ethiopia and found that *Aspergillus* species (75 %), *Fusarium* (11 %), *Penicillium* (8 %) and *Trichoderma* (6 %) were the most abundant genera in the maize. Another study found Aflatoxins which is produced by *Aspergillus* or *Penicillium* and fumonisins produced by *Fusarium* in most locally grown maize in 18 African nations during 2007 and 2008 (Probsr et al., 2014). Lane et al. (2018) also found that *Fusarium* was the most abundant genus after exploring the fungal microbiome of corn during hermetic storage the United States and Kenya.

Several techniques have been applied to decontaminate food crops from fungi and associated mycotoxins. Infrared (IR) heating has gained popularity over decades for its versatile

applications including baking, drying, thawing, blanching, and microbial decontamination (Pan and Atungulu, 2010). IR heating leads to thermal denaturation of proteins and nucleic acids in microorganisms and thereby inactivate the microbes (Ramaswamy et al., 2012). In 2014, IR heating was used to decontaminate *A. flavus* on rice grains in a shorter heating duration than convective methods (Wang et al., 2014). Wilson et al. (2017) achieved a 4.03 log (CFU/g) and 4.27 log (CFU/g) fungal load reduction in high MC corn (24% wet basis) after one-pass IR heating plus tempering at 50°C for 4h and two passes IR hating plus tempering at 70°C for 2h, respectively . Researches have shown that the total load of fungi decreased significantly by exposing the corn samples to IR heating. However, there is limited information on diversity of fungi that are susceptible to the processing (Wilson et al., 2016 & 2017). It was reported that the degree of sensitivity varied among different isolates of fungi; meaning that some species may be more resistant to inactivation and some may not (Pereira et al., 2013).

To evaluate efficiency of decontamination methods, there are several methods used to analyze microbial communities. The conventional methods that have been used for years are time consuming, labor intensive, and complex. Also, the traditional methods are not able to identify all the fungi species present in a food system (Espy et al., 2006). The advanced identification methods such as molecular techniques are able to identify different type of fungi. The molecular methods identifies fungi based on ribosomal DNA (rDNA) sequences (Iwen et al., 2002). The information obtained from DNA sequences is translated and analyzed into taxonomic groups of fungi. The important molecular target for identification of fungal taxonomy has been introduced as intervening internal transcribed spacer (ITS) regions (Iwen et al., 2002). Currently, high-throughput sequencing and metagenomics studies have been used as a powerful tool to study fungi communities in food (Ercolini, 2013). The Illumina MiSeq is a platform of high-

throughput sequencing that provides sequences information at greater depth compared to other techniques. Illumina was recognized as a fully feasible and promising approach to characterize soil fungal community and recover fungal richness (Schmidt et al., 2013). The Illumina Miseq has the possibility of detecting dead fungi but due to the high temperature associated with IR and tempering process it likely that the dead fungi cell was completely destroyed. By saying so, identification of fungal sensitivity to selective IR-heating wavelength necessitates the use of modern techniques such as Illumina MiSeq.

The objectives of the current study are 1) identification of how fungal communities respond to IR heating; 2) the feasibility of IR heating to inactivate mycotoxigenic fungi, such as *Aspergillus.*

Materials and methods

The Corn samples, infrared heating device, measurement of radiant energy transfer, and experimental design that was used in this study is outlined in chapter 3 (pp.30 - pp.33).

Genomic DNA extraction

A 10 g sample of corn was mixed with 90 mL phosphate-buffered dilution water in a sterile stomacher bag and masticated. The solution was centrifuged at 10,000 × g for 10 mins at 4°C. After centrifuging the solution was thrown away leaving a pellet in the bottom of the tube. Genomic DNA was extracted from the corn pellet using DNeasy Plant Pro kit (Geneaid, USA).

Polymerase Chain Reaction (PCR)

PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand (Maddocks and Jenkins, 2017). PCR reactions (25 µL) contained 2.5 µL of Buffer II, 0.1 µL of AccuPrime™ Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, United States), 3 µL of DNA, and 1 µL of each dual-index primer combination. Each primer (ITS1 and ITS2) consisted of the appropriate Illumina adapter (AATGATACGGCGACCACCGAGATCTACAC for ITS1 and CAAGCAGAAGACGGCATACGAGAT for ITS2), an 8-nt index sequence (each index being different from each other), a 10-nt pad sequence (TGTGGTGGCC for ITS1 and ACTGCGTCAT for ITS2), a 2-nt linker (GT for ITS1 and AT for ITS2) and the gene-specific primer (CTTGGTCATTTAGAGGAAGTAA and GCTGCGTTCTTCATCGATGC for ITS1 and for ITS2, respectively). The PCR was performed at 94°C for 2 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 1 min; and a final extension at 72°C for 5min

Sequencing

The PCR products were normalized and pooled using the SequalPrep Normalization Plate Kit (Applied Biosystems™, Waltham, MA, USA). After constructing the DNA library, the Illumina Miseq at the Biomass Research Center (BIOR), University of Arkansas was used to sequence the amplified ITS region using a Miseq v2 500 cycle kit (Illumina Inc., San Deigo, CA, USA). A total of 1,328,549 reads were obtained after sequencing.

Bioinformatics

The data generated from Illumina MiSeq sequencing was analyzed for its fungal biodiversity using PIPITS (Gweon et al., 2015). PIPITS is a user-friendly computational tool for automated processing of large sequence data sets from quality filtering to generation of species abundance table of fungal ITS sequences (Gweon et al., 2015). There are three elements of the PIPITS pipeline, namely PIPITS PREP, which prepares raw reads for the extraction of ITS (ITS1 or ITS 2), PIPITS FUNITS, which extracts fungal ITS subregion from the reads, and PIPITS PROCESS, which analyzes reads for the production of operational taxonomic unit (OTU) tables (Gweon et al., 2015). The pipeline process is shown in Figure 1.

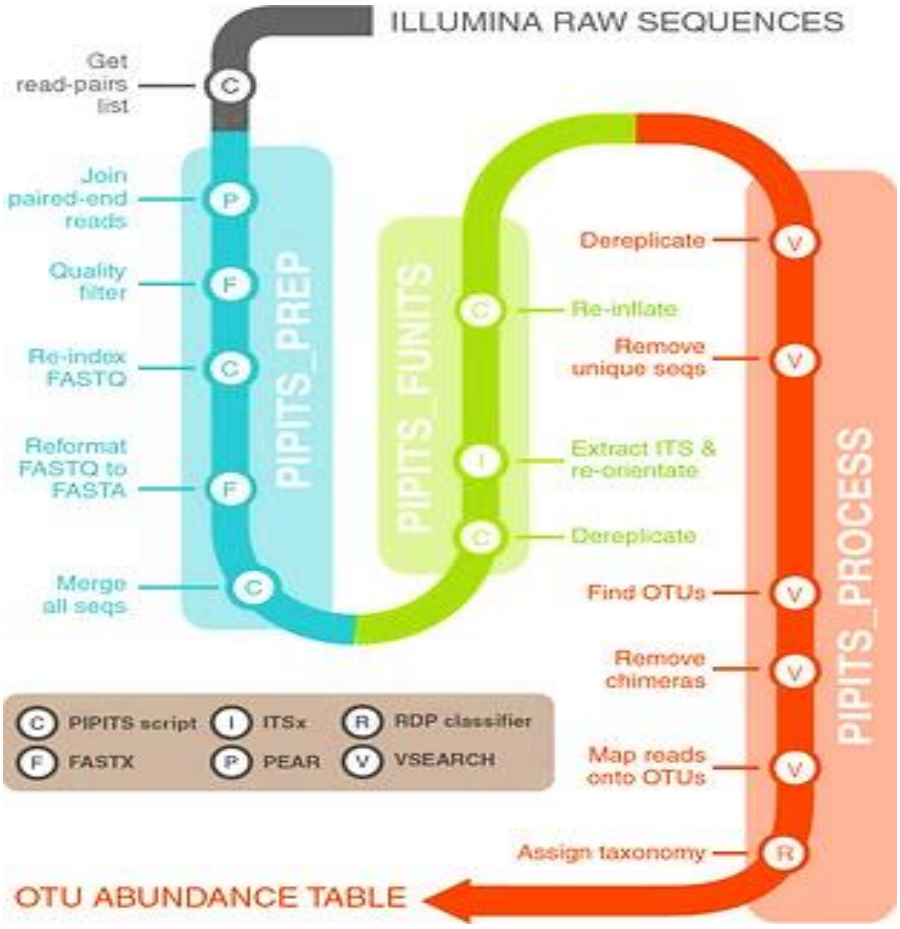

Fig. 1. Outline of PIPITS for Illumina Internal transcribed spacer sequences

Statistical analysis

Analysis of variance (ANOVA), RSM, and principle component analysis (PCA) were performed with a statistical software (JMP version 14.0.0, SAS Institute) to determine significant differences within and among samples. The RSM was used to describe the response-factor relationship. Level of significance (α) was set at 5% for comparing means.

Results and discussion

Fungal community structure analysis after selective infrared (IR) treatments

The major phyla for IR treated samples without tempering (non-tempered) are shown in Figure 2. The most abundant phylum for the non-treated corn samples (control) with MC of 16%, 20%, and 24% w.b. was Ascomycota followed by unclassified and then Basidiomycota, respectively (Figure 2). The most abundant phylum for non-tempered samples was Ascomycota with 95.6% of the sequences on average followed by Basidiomycota which represented 0.6% (Figure 2). There was a percentage of 3.8 as a not classified at the phylum level. For IR treated samples followed by a tempering step (Figure 3), the most abundant phylum was Ascomycota with 93.2% of the sequences on average followed by not classified ones (5.9%), and then Basidiomycota (0.84%). Similar results were reported by Abe et al. (2015), where fungi isolated from maize (Zea mays L.) grains were explored. Abe et al. (2015) found that 91.31% of the isolates were *Ascomycota*, Zygomycota (4.34%) and Basidiomycota (4.34%).

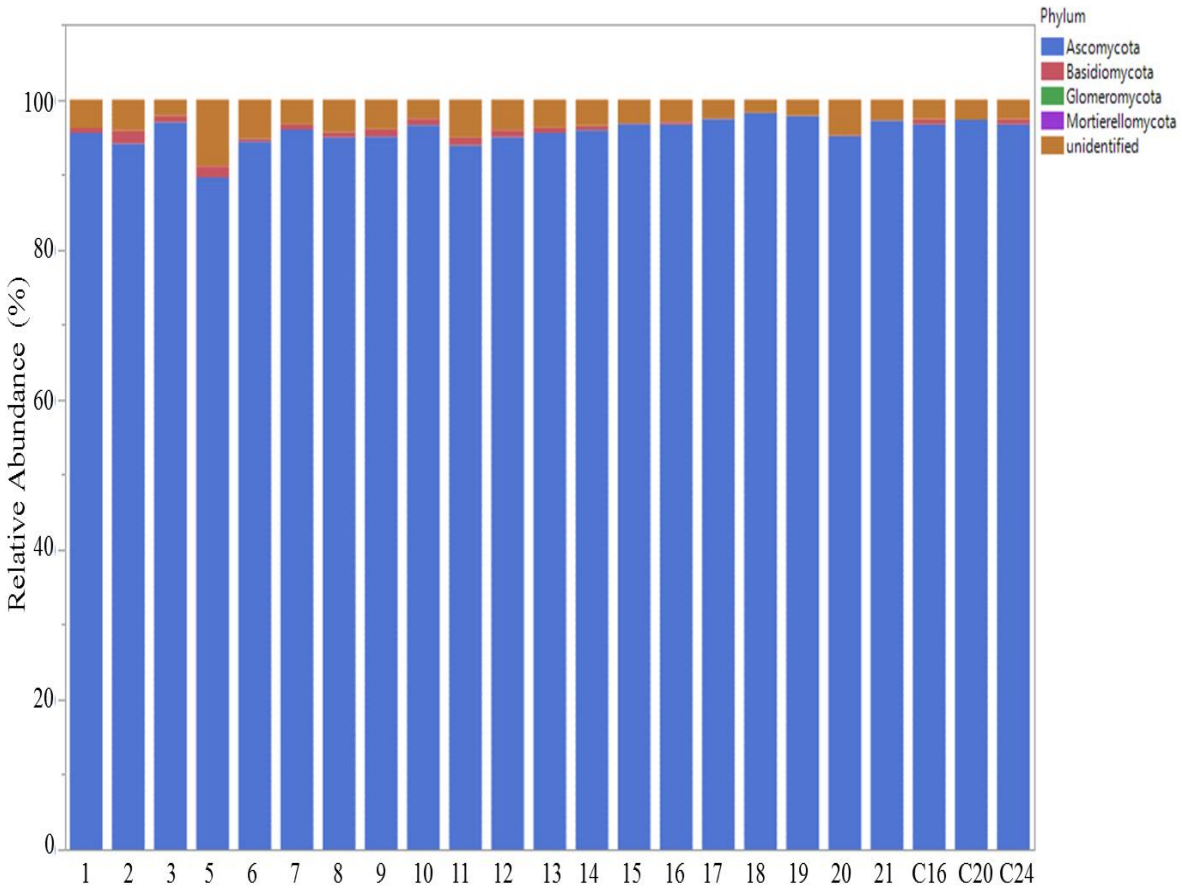

Fig. 2. Percentages of the major fungal phyla on corn after selective infrared heat treatments; C16, C20, and C24 signifies the relative abundance of non-treated (control) corn samples with moisture content of 16%, 20%, and 24% wet basis, respectively.

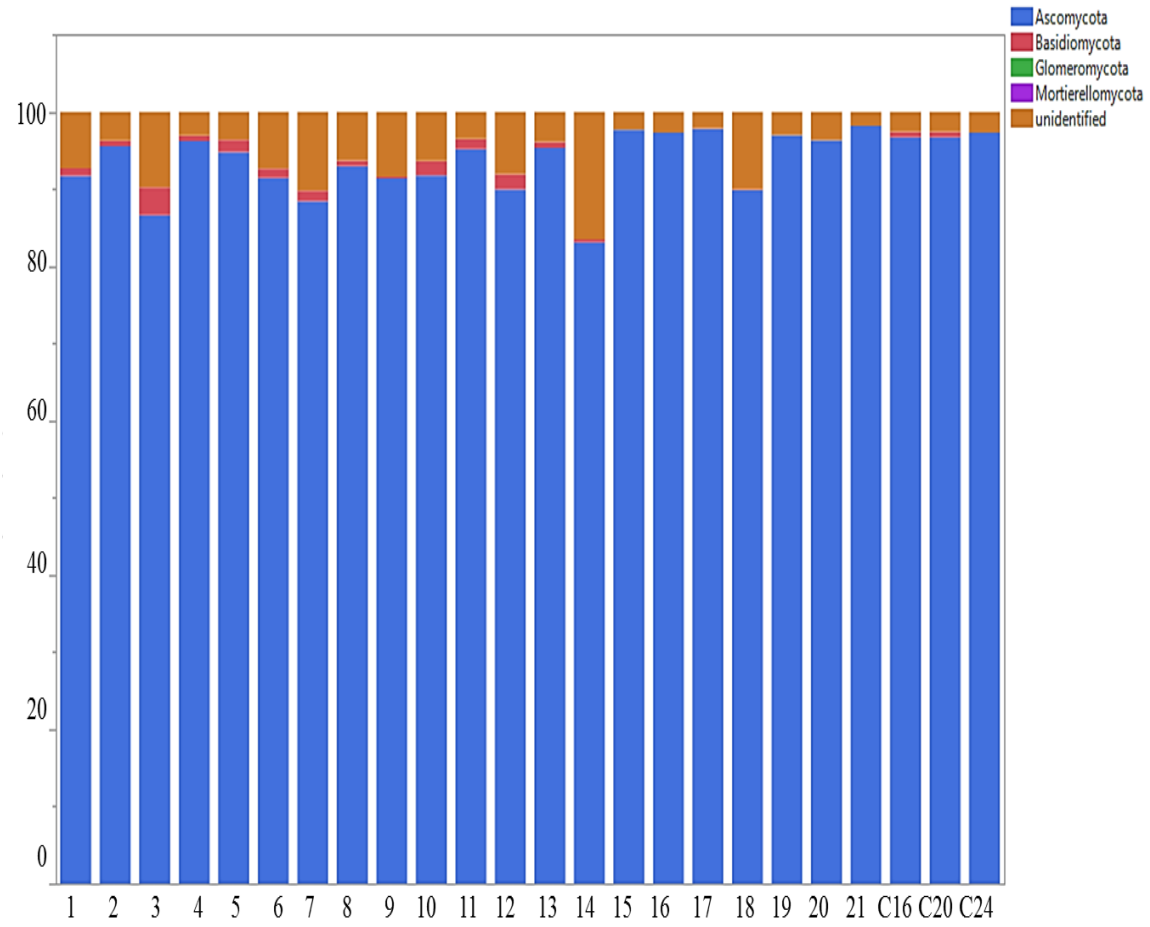

Fig. 3. Percentages of the major fungal phyla on corn after selective infrared and tempering heat treatments; C16, C20, and C24 signifies the relative abundance of non-treated (control) corn samples with moisture content of 16%, 20%, and 24% wet basis, respectively.

The major genera found in corn samples after selective IR treatments are in summarized in Figure 4 and Figure 5 for samples treated with IR followed by no tempered and tempering, respectively. For both non-tempered and tempered samples, mycotoxin producing fungi were identified. *Aspergillus* was the primary genus found on non-tempered samples while *Meyerozyma* was the primary genus for tempered samples. As it is reported by other studies, some of the mostly discovered genera in corn were *Aspergillus, Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma* (Bhattacharya and Raha, 2012; Niaz and Dawar, 2009; Ekwomadu et al., 2018). Askun, (2006) found that in corn, *Fusarium* (82%), *Penicillium* (63%), and

Aspergillus species (33%) were most predominant compared to the other genera. Askun, (2006) also discovered that *Aspergillus* (19%) was one of the most common genera isolated from disinfected and non-disinfected corn kernels.

	Treatments																					
Genus	C16	1	2	3	4	5	6	7	C20	8	9	10	11	12	13	14	C24	15	16	17	18	19	20	21
Aspergillus	72.76	48.08	74.22	50.75	42.54	62.75	51.16	48.16	72.76	64.71	82.97	80.14	80.99	83.39	83.62	75.63	0.02	0.24	0.06	0.35	0.17	0.06	0.42	0.09
Candida	0.59	0.67	1.56	0.40	3.73	0.47	1.40	2.66	0.59	0.92	0.85	1.41	0.82	0.89	0.78	3.72	0.34	1.04	0.91	1.00	0.75	1.44	2.76	0.72
Meyerozyma	0.74	2.82	2.98	1.19	5.04	2.12	1.59	5.28	0.74	1.33	1.53	1.24	1.66	1.98	1.55	3.30	26.49	56.70	66.38	49.08	60.64	43.50	55.73	29.79
Papiliotrema	0.17	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.17	0.81	0.94	0.19	0.23	0.37	0.14	0.45	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Penicillium	1.01	0.03	0.05	0.01	0.04	0.03	1.95	0.06	1.01	3.13	1.60	1.32	1.91	1.53	2.14	1.42	3.74	0.29	0.00	0.00	0.03	0.01	0.06	0.02
Phialemoniopsis	0.00	1.13	1.25	2.18	0.79	0.80	1.29	1.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04	0.05	0.26	0.06	0.05	0.17	0.12
Pyxidiophora	0.00	0.01	0.18	0.12	0.02	0.02	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.15	0.09	0.19	0.12	0.20	0.29	0.30
Sarocladium	0.07	0.05	0.09	0.06	0.04	0.08	0.06	0.05	0.07	0.05	0.12	0.07	0.20	0.16	0.15	0.20	0.01	0.29	0.07	0.20	0.12	0.08	1.34	0.26
Talaromyces	1.57	0.03	0.13	0.03	0.45	0.02	0.02	0.05	1.57	0.14	0.19	0.12	0.32	0.46	0.21	0.14	0.00	0.04	0.04	0.12	0.03	0.05	0.11	0.06
Trichoderma	0.00	0.31	4.78	0.47	1.04	0.28	0.68	1.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.71	0.17	0.35	0.18	0.00	0.89	0.12
unidentified	0.21	46.13	12.73	44.23	45.04	31.74	41.36	39.64	0.21	1.71	1.32	0.72	0.77	2.34	2.09	0.89	21.37	38.93	30.96	48.15	23.45	54.04	36.70	67.92
Valsaria	22.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.00	26.30	9.43	13.61	11.76	7.77	8.43	13.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wallemia	0.55	0.58	1.62	0.35	0.22	1.41	0.33	0.99	0.55	0.54	0.61	0.66	0.77	0.67	0.48	0.45	0.01	0.06	0.02	0.02	0.02	0.03	0.03	0.03
Wickerhamomyces	0.00	0.03	0.01	0.02	0.13	0.10	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	47.75	1.42	0.90	0.19	14.23	0.49	1.33	0.52
Zygoascus	0.04	0.01	0.10	0.03	0.85	0.03	0.02	0.04	0.04	0.04	0.03	0.11	0.08	0.06	0.04	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Fig. 4. Percentages of the major fungal genera on corn after selective infrared heat treatments. C16 signifies the relative abundance of non-treated (control) corn samples with moisture content of 16% wet basis; C20 signifies the relative abundance of non-treated (control) corn samples with moisture content of 20% wet basis; C24 signifies the relative abundance of non-treated (control) corn samples with moisture content of 24% wet basis.

Genus	C16	1	2	3	4	5	6	7	C20	8	9	10	11	12	13	14	C24	15	16	17	18	19	20	21
Arthrinium	0.05	0.07	0.03	0.03	0.00	0.32	0.10	0.04	0.05	0.04	0.02	0.02	0.09	0.15	0.18	0.28	5.14	3.34	4.85	4.23	3.38	4.71	5.39	3.19
Aspergillus	72.76	1.19	0.65	0.66	0.56	0.83	1.29	0.79	72.76	0.84	0.92	1.19	0.65	0.77	0.47	0.93	0.02	0.57	0.40	0.25	0.84	0.41	0.10	0.37
Candida	0.59	0.34	0.17	0.05	0.09	0.08	0.23	0.04	0.59	3.04	0.15	0.03	0.14	0.05	0.05	0.09	0.34	0.18	0.04	0.01	0.07	0.03	0.03	0.02
Cladosporium	0.02	0.13	0.08	0.01	0.03	0.07	0.10	0.11	0.02	0.65	0.02	0.03	0.01	0.03	0.14	0.01	1.70	1.34	1.57	3.78	1.94	2.08	2.49	4.11
Meyerozyma	0.74	8.47	2.27	1.36	21.77	3.79	9.67	3.76	0.74	17.61	29.64	1.72	5.34	6.94	2.51	6.51	26.49	26.19	36.00	33.84	28.04	25.76	24.24	21.74
Nigrospora	0.00	5.38	2.40	0.74	1.96	1.41	3.73	2.14	0.00	1.31	0.74	1.40	0.94	1.50	2.17	4.62	0.07	0.15	0.47	0.07	0.18	0.10	0.17	0.14
Penicillium	1.01	2.03	9.23	2.61	10.96	1.60	0.57	0.80	1.01	1.52	2.19	4.87	8.65	0.64	1.44	0.74	3.74	0.58	0.70	1.39	8.98	5.39	0.87	0.91
Pyxidiophora	0.00	0.64	0.10	0.08	0.46	0.19	0.09	0.08	0.00	0.09	0.31	0.90	3.56	0.39	0.16	4.26	0.06	0.10	0.09	0.01	0.06	0.37	0.07	0.03
Rhinocladiella	0.01	0.24	0.13	0.04	0.01	0.06	4.46	0.08	0.01	1.24	0.17	0.20	0.42	0.06	1.67	0.02	0.01	0.00	0.01	0.02	0.14	0.62	0.05	0.04
Saccharomyces	0.01	0.60	0.45	6.61	0.37	0.67	0.39	0.38	0.01	0.51	0.40	0.62	0.56	0.37	0.33	0.31	1.29	0.29	0.15	0.10	0.20	0.16	0.07	0.36
Trichoderma	0.00	0.92	9.23	0.12	0.37	1.02	5.43	1.24	0.00	0.52	1.15	0.40	0.18	1.45	0.48	0.17	0.01	0.13	0.02	0.07	0.35	0.02	0.06	0.02
unidentified	0.21	74.83	73.79	87.06	61.68	88.54	72.22	89.00	0.21	71.22	63.22	86.49	78.03	85.26	87.99	78.88	21.37	65.77	55.22	55.97	55.10	59.91	66.06	68.55
Wickerhamomyces	0.01	3.06	0.63	0.11	0.41	0.48	0.65	0.28	0.01	0.52	0.21	1.11	0.67	1.17	1.61	1.43	47.75	0.00	0.00	0.01	0.01	0.00	0.00	0.01

Fig. 5. Percentages of the major fungal genera on corn after selective infrared and tempering heat treatments; C16, C20, and C24 signifies the relative abundance of non-treated (control) corn samples with moisture content of 16%, 20%, and 24% wet basis, respectively.

Niaz and Dawar (2009) reported that corn in Pakistan was contaminated with a variety of fungi; *Aspergillus flavus, A. niger, A. wentii* and *Penicillium* spp were the most predominant ones found in 70% of the examined corn samples (Niaz and Dawar, 2009). In some other studies, different species of *Fusarium* spp (Desjardins et al., 2000; Macdonald et al., 2005). Also, it was confirmed by Ghiasian at al. (2004) that corn was infected mainly by *Fusarium* spp (38.5%) followed by *Aspergillus* (8.7%), *Rhizopus* (4.8%), *Penicillium* (4.5%), *Mucor* (1.1%), and other fungal genera (Ghiasian at al., 2004). Mycotoxigenic fungi prevalence on corn is a global problem.

Feasibility of selective IR heating to inactivate mycotoxigenic fungi

Analysis was performed to investigate the correlation between selected peak wavelength, heating duration, MC, and PEG on the microbial load reduction after IR and tempering treatments. After IR treatment, the effects of selected peak wavelength, heating duration, MC, and PEG had a statistically significant effect on the relative abundance of the *Aspergillus* and *Penicillin* ($p < 0.0001$) (Table 1 and Table 2). The effect of IR treatment combined with tempering was not significant. The model explained 78% and 84% of the variation for *Aspergillus* and *Penicillin* for non-tempered samples, respectively.

Table 1. Effect test table showing the effects of selective infrared heat treatment on the relative abundance of *Aspergillus* for non-tempered corn samples.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	14	69871.341	4990.81	17.5008
Error	48	13688.484	285.18	**Prob > F**
C. Total	62	83559.825		<.0001

Table 2. Effect test table showing the effects of selective infrared heat treatment on the relative abundance of *Penicillium* for non-tempered corn samples.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	2	32.666965	16.3335	144.0821
Error	57	6.461651	0.1134	**Prob > F**
C. Total	59	39.128616		<.0001

The RSM analyses were performed to investigate the correlation between the selected peak wavelength, heating duration, MC, and PEG on the reduction of A. *flavus* after IR treatments. The main effect of MC and the quadratic effect of MC had a statistically significant effect on the *Aspergillus* and *Penicillin* ($p > 0.05$) genera (Table 3).

Table 3. Effect summary table showing the effects of moisture content on the relative abundance of Aspergillus and Penicillium (%).

	PValue	
Source	**Aspergillus**	**Peniccilum**
Moisture Content (%w.b.)* Moisture Content (%w.b.)	0.00000	<.0001
Moisture Content (%w.b.)	0.00000	0.4463

The results were confirmed by PCA (Figure 6) based on the diversity of each fungal community. As it is shown in Figure 6, the principal components 1 and 2 account for most of the variation in the data. Component 1 accounts for 75.4% and Component 2 accounts for 24.6% variations in the data. So, the PCA using component 1 and 2 here explains the model very well. As shown in Figure 6, the three MC tested were clearly separated.

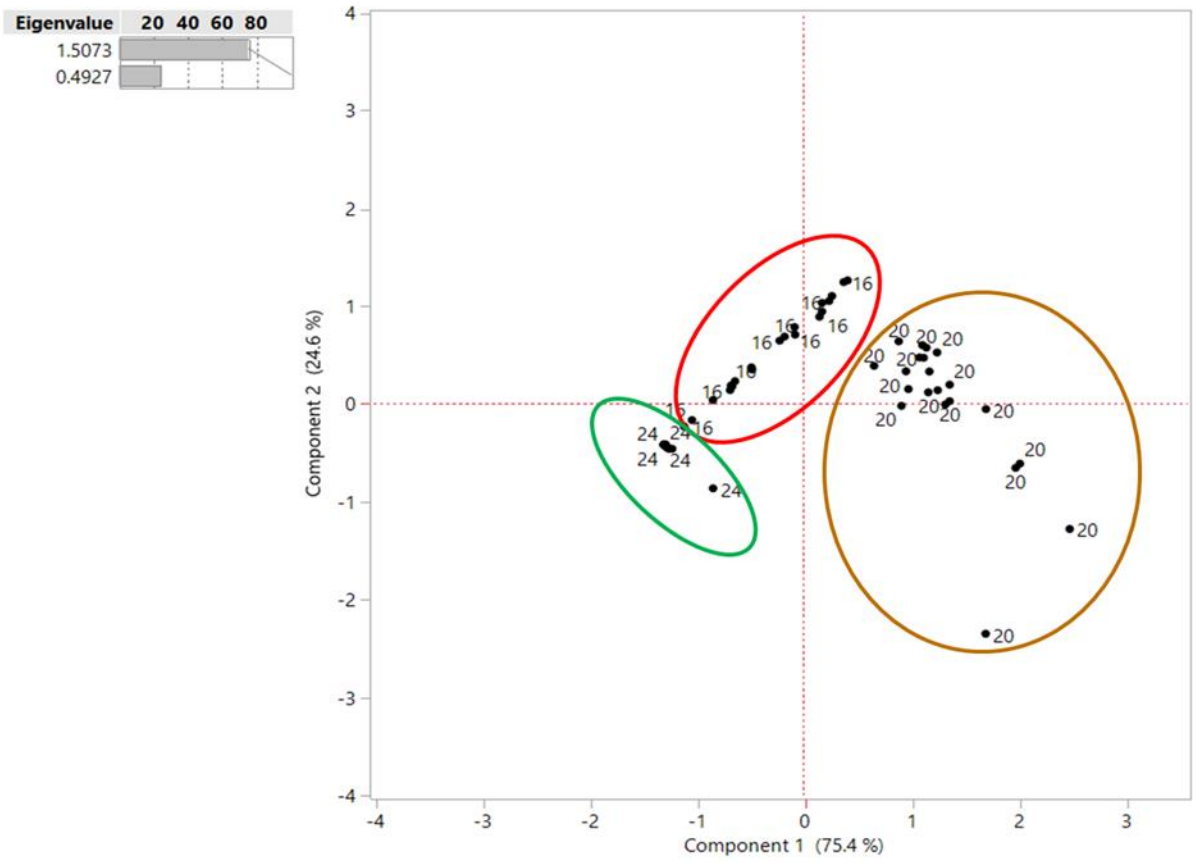

Fig. 6. Principal component analysis (PCA) explaining selective infrared treatment effect on relative abundance of *Aspergillus* and *Penicillium* at different moisture content (16%, 20%, and 24% wet basis).

Jun et al. (2003) found significant effects of irradiation parameters such as emitter gap distance, irradiation duration, and input voltage when inoculated corn with *Aspergillus niger* was irradiated with UV-pulsed light. The authors by doing RSM found that the logarithmic reduction of *A. niger* followed a curvilinear trend specially by increasing treatment duration, input-voltage, and decreasing the emitter distance (Jun et al., 2003). Impact of IR and tempering conditions also was reported by Wilson et al. (2017). The results obtained from their study showed that tempering was able to reduce fungi significantly on IR-treated corn. Also, increasing tempering duration from 2 h to 6 h resulted in an average of 63.5% reduction of fungi population on corn (Wilson et al., 2017).

Conclusion

This study examined the use of IR treatments followed by tempering to decontaminate fungal community on the grain. The findings showed that after treating corn samples at different selective wavelengths, heating duration, PEG and MC, Ascomycota was the most dominant phylum for non-tempered and tempered treatments. Some treatment combinations were able to reduce the abundance of mycotoxin producing fungi such as *Aspergillus* and *Penicillium*. For example, a sample with MC of 16% w.b. that was treated at PEG of 275 mm and wavelength of 4.5μm for 60 s was able to reduce the relative abundance of *Aspergillus* from 72.8% to 42.5%. After the IR treatments it was noted that the fungal community was separated by the different corn MCs. Selective IR treatments showed promise as an approach to improve inactivation of fungi such as of *Aspergillus* genus which are known to produce toxins on grains.

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Chapter 5: Impact of selective infrared wavelengths on fungal decontamination of shelled corn inoculated with *Aspergillus flavus*

Abstract

Selective infrared (IR) heating was explored as technique for decontaminating *Aspergillus flavus* (*A. flavus*) mold spores on shelled corn**.** The objective of this study was to determine the suitable combinations of IR heating duration, intensity, wavelengths, and moisture content (MC), followed by tempering treatments to maximize decontamination of *A. flavus* mold spores. Corn samples were inoculated with spore suspension of *A. flavus* and incubated to allow microbial attachment on the kernel. Corn samples were then heated using IR energy then tempered at 70ºC for 4 h. Treatments at wavelength of 3.2 µm, product-to-emitter-gap sizes (PEG) of 110 mm and corn MC of 24% wet basis (w.b.) for non-tempered and tempered samples resulted in the greatest *A. flavus* load reduction of 4 Log CFU/g. The higher the corn MC, IR intensity and heating duration the less fungal regrowth was observed after IR and tempering treatments. From the results of the present study, IR treatments could improve corn quality and safety, thereby improving returns to producers and processors.

Keywords: Selective infrared heating, selected peak wavelengths, fungi, product-to-emitter-gap sizes, *Aspergillus flavus*

Introduction

Corn production plays a major role in the U.S. economy (Prasad et al., 2018). With 96,000,000 acres (39,000,000 ha) of land reserved for maize production; the US is the world's largest maize producer. The United States grew more than 14.6 billion bushels (385 million metric tons) of maize in the 2016/2017 crop marketing year and approximately 17% of production has been exported to over 80 countries (U.S. GRAINS COUNCIL, 2019). Corn is also processed into different food and industrial products including starch, sweeteners, corn oil, beverage and industrial alcohol, and fuel ethanol (USDA, 2018).

Recently, priority has been given to food storage and preservation to enhance global food security. During storage, qualitative and quantitative losses are mainly caused by microbial infection and infestation of pests (Pande et al., 2012). Contamination of grain with mold spores has always been a challenging issue in the food industry. *Aspergillus flavus, Fusarium* spp., and *Penicillium* spp. are common pathogenic fungi associated with stored corn kernel (Pechanova and Pechan, 2015). *A. flavus* is known to produce aflatoxin and have received the most attention due to potent carcinogenic and acute toxicological effects in humans (Williams et al., 2004; Khlangwiset et al., 2011; Faulkner, 2014). If aflatoxin is consumed by humans and animals, it can lead to suppressed immune response, malnutrition, bile duct proliferation, centrilobular necrosis, liver infiltration, hepatic lesions, impairment of growth, feed refusal, emesis and even hepatomas (Williams et al., 2004; Khlangwiset et al., 2011; Bbosa et al., 2013; Hussein and Brasel, 2001).

Some post-harvest strategies for prevention of harmful fungi include improved drying methods, good storage conditions, irradiation, the use of natural and chemical agents, limiting physical damage of grains during harvesting, and avoiding insect infestation (Mohapatra et al.,

2017; Ukwuru et al., 2018). For safe storage of grains, chemical treatments such as fumigation with ethylene oxide, propylene oxide and ethyl and methyl formate are being used. The deficiencies with these methods include high costs, the development of resistant strains and the problem of chemical residues, which can affect humans (Gulati and Mathur, 1995). Thus, researchers are paying considerable attention to alternative treatments for safe storage of grains.

IR radiation has also been used as a non-chemical method for decontamination of food grains. IR is a part of the electromagnetic spectrum and can be classified as Near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR) and ranges 0.75 to 1.4, 1.4 to 3, and 3 to 1000 μm, respectively (Krishnamurthy et al., 2008). Compared to conventional methods, IR treatment has greater advantages such as high degree of process control parameters, improved energy efficiency and uniform product temperature, resulting in better-quality finished products (Krishnamurthy et al., 2008; Rastogi, 2012).

IR treatment was used to inactivate pathogenic fungi *A. niger* in corn meal, resulting in a reduction of 2.3 $\log_{10}$ CFU/mL in 6 minutes (Jun and Irudayaraj, 2003; Krishnamurthy et al., 2008). Researchers also investigated the decontamination of foods such as strawberry, wheat, corn flour, grains, cottage cheese using IR radiation (Rosenthal et al., 1996; Jun and Irudayaraj, 2003; Hamanaka et al., 2006; Rastogi, 2012; Wilson et al., 2017). Based on these facts, the current work focuses on exploring the effect of selective IR treatment on decontamination of corn inoculated with *A. flavus* spores.

Materials and methods

The Corn samples, infrared heating device, measurement of radiant energy transfer, and experimental design that was used in this study is outlined in chapter 3 (pp.30 - pp.33).

Inoculation of shelled corn

A. flavus strain ATCC 28539TM (American Type Culture Collection, Manassas, VA) were procured in vials. The contents of each vial were mixed with 1.0 mL of sterile water; then transferred to a test tube containing 6 mL of sterile water. *A. flavus* was allowed to rehydrate for 3 h and cultured on potato dextrose agar (PDA). After 7 days of incubation at 25^{o}C, spores were detached by flooding the culture plates with 0.03% Tween 80. Initial volume with detached spores was designated the original inoculum concentration. Samples were inoculated with the original inoculum of *A. flavus* spore suspension in a sterilized Erlenmeyer flask. The flasks were then covered with aluminum foil and kept in an incubator for 5 days at 35^{o}C. To achieve uniform fungal attachment, the flask containing corn kernels were manually shook every 12 h.

Aspergillus flavus enumeration

Rose Bengal Agar (RBA) supplemented with dichloran and a stock solution of streptomycin and chlortetracycline plates were used to enumerate *A. flavus* counts. RBA is a selective medium used to detect and enumerate yeasts and molds in food samples. After medium is cooled, 0.1 mL aliquots of sample solution were spread on the Petri plates using glass hockey sticks. The RBA plates were incubated (Thelco Model 4, Precision Scientific Instruments, Inc., Chicago, IL) at 25°C for 120 h before counting. After incubation, the colony forming units (CFU) on each plate were counted.

Fungal regrowth

To examine the potential for mold regrowth after IR treatments, treated corn was placed in favorable conditions for mold growth (33ºC, and 90% RH). This regrowth environment was created using saturated Potassium chloride (KCl) solution. Treated corn was placed in a cheese cloth then suspended in a jar above a salt solution of 80 g KCl and 100 mL H_2O. Corn samples were incubated at 33ºC for 5 days.

Statistical analysis

Analysis of variance (ANOVA) and RSM were performed with a statistical software (JMP version 14.0.0, SAS Institute) to determine significant differences within and among samples. The RSM was used to describe the response-factor relationship. All tests were considered to be significant when $p < 0.05$.

Results and discussion

Implications of selective infrared heating and tempering treatments on *Aspergillus flavus*

The least square mean of the *A. flavus* concentration of control samples were 5.0 ± 0.5, 5.2 ± 0.3, 5.1 ± 0.6 Log CFU/g for corn with initial MC of 16%, 20%, and 24% w.b., respectively. The effect of wavelengths, treatment durations, IR intensities, and corn MC on inactivation of *A. flavus* for non-tempered and tempered corn samples are presented in Figure 1 and Figure 2. Treatments at higher heating duration, lower PEG and lower wavelength lead to greater reduction of *A. flavus* spores (Figure 1). Similar trends were observed for tempered samples. When the tempering step was added, *A. flavus* concentration was < 2.5 Log CFU/g for all treatments. Hamanaka, et al. (2010) suggest that the decontamination provided by IR

treatments may be caused by the denaturation of essential proteins and damage to nucleic acids, cell walls, and cell membranes of fungi.

As the corn MC level increased, the *A. flavus* concentration decreased (Figure 2). Research has shown that the thermal destruction of fungal colonies provided by IR treatments is greater in agricultural commodities with high MCs (>22%) than in those with lower MCs (Sandeep, 2011; McCann et al., 2009). McCann et al. (2009) and Staack et al. (2008) also found that high water activity can substantially enhance the decontamination of spores after heat treatments.

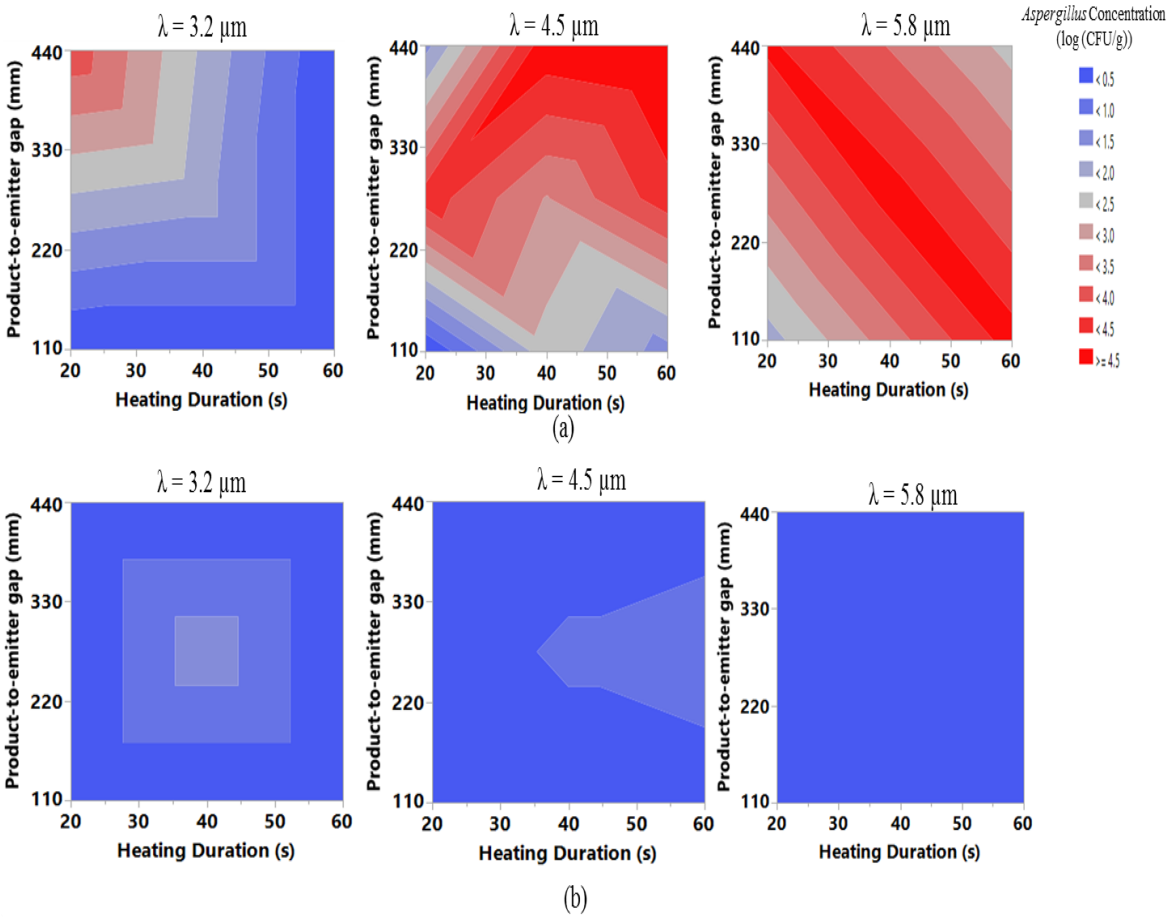

Fig. 1. Contour plots of *Aspergillus flavus* concentration (log CFU/g) as influenced by the interaction between heating duration (s), wavelength (µm) and product-to-emitter gap size (mm) for non-tempered (a) and tempered treatments (b), respectively; CFU means colony forming units; λ signifies wavelength in µm.

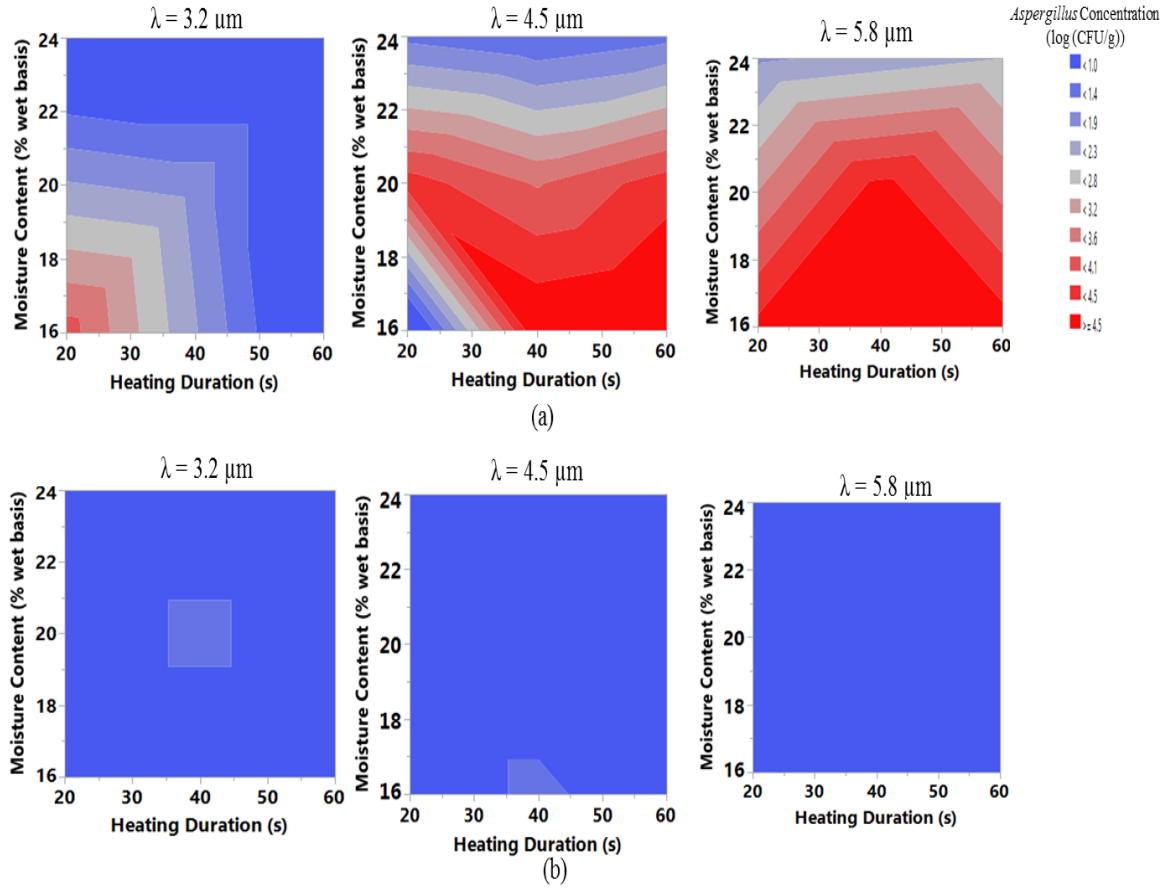

Fig. 2. Contour plots of *Aspergillus flavus* concentration (log CFU/g) as influenced by the interaction between heating duration (s), wavelength (µm) and corn moisture content (% w.b.) for non-tempered (a) and tempered treatments (b), respectively; CFU means colony forming units; λ signifies wavelength in µm.

Generalized regression analyses were performed to investigate the correlation between the selected peak wavelength, heating duration, MC, and PEG on the reduction of A. *flavus* after IR and tempering treatments. After IR treatment, the effects of selected peak wavelength, heating duration, MC, and PEG had a statistically significant effect on the A. *flavus* concentration ($p < 0.05$). The model explained 78 % of the variation for non-tempered samples. After tempering treatments, the *A. flavus* concentration reduced significantly compared to the control but no statistical difference was observed between the treatment combinations ($p > 0.05$).

Table 1 shows the effect summary table for the *A. flavus* concentration response for non-tempered samples. The effect summary table for the *A. flavus* response indicates high statistical significance ($p<0.05$). The supplied wavelength ($p<0.0001$), PEG ($p<0.0001$) and corn MC ($p<0.0001$) significantly impacted the *A. flavus* concentration as indicated by the effect test (Table 1). There was a statistically significant interaction between the wavelength and the PEG ($p = 0.0008$). There was also a quadratic statistically significant effect between MC ($p<0.0001$) for non-tempered treatments.

Table 1. Effect summary table showing the effects of moisture content, wavelength and product-to-emitter gap on *Aspergillus flavus* concentration (Log (CFU/g-grain)

Term	**Prob > ChiSquare**
Moisture Content (% w.b.)(16,24)	<.0001*
Moisture Content (% w.b.)*Moisture Content (% w.b.)	<.0001*
Product-to-emitter gap (mm)(110,440)	<.0001*
Product-to-emitter gap (mm)*Wavelength (μm)	0.0008*
Wavelength (μm)(3.2,5.8)	<.0001*

Optimization of *Aspergillus flavus* load reduction

The factors in this experiment have combinatorial nature because within each factor there are different levels. Therefore, the process must be optimized to determine the best combinations of wavelength, MC, heating duration, and product-to-emitter gap size needed to achieve the greatest responses in terms of lowest mold load. According to the prediction profiles, it was determined that minimum levels of microbial load could be obtained at factor settings shown in Figure 3 for IR treatments. A wavelength of 3.2 μm, PEG of 110 cm and MC of 24% w.b. are

preferred for optimum inactivation of mold load on corn. Figure 3 also provided a desirability of 0.90 which implies that approximately 90% of the goals to minimize corn mold count were achieved.

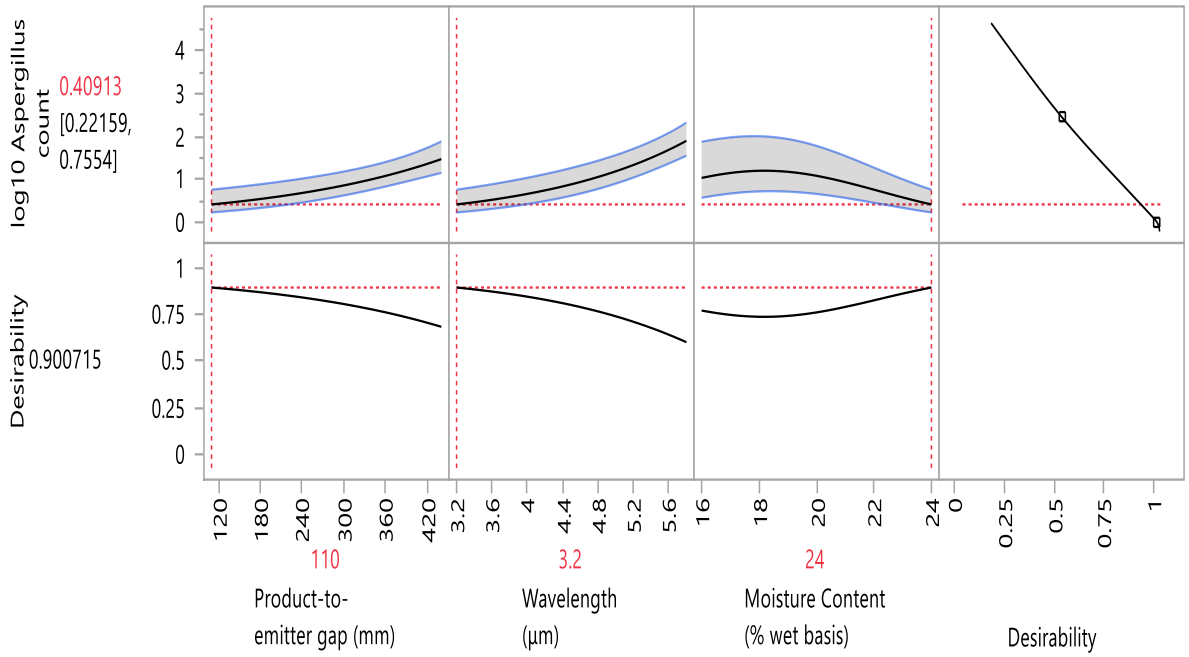

Fig. 3. Prediction profile for *Aspergillus flavus* concentration (Log (CFU/g-grain)) responses with parameter settings product-to-emitter gap size (mm), wavelength (µm) and corn moisture content (% w.b.) for non-tempered treatments.

Effect of selective infrared heating and tempering treatments on the regrowth of *Aspergillus flavus* spores

The implications of increasing IR treatment duration and adding a tempering step on *A. flavus* concentration after being subjected to conditions favorable for regrowth for non-tempered (a) and tempered (b) corn samples can be seen in Figure 4. The concentration of *A. flavus* decreased with increasing doses of IR treatment. Decreasing IR wavelength and increasing treatment duration supplied for both the tempered and non-tempered corn samples resulted in statistically significant decreases in *A. flavus* concentration as indicated by the effect test (Figure

4). It was noted that at higher corn MC there was an increase in *A. flavus* regrowth. Also, as the heating duration increased, the *A. flavus* count decreased. There was an increase in the *A. flavus* concentration after the regrowth for the non-tempered treatments.

The decontamination is further improved when a tempering step is added as the high RH associated with the step contributes to increased decontamination. Although, there was apparent decontamination of *A. flavus* mold spores, there was apparent mold regrowth at treatments with the lower IR intensity and higher MC. This is probably due to the survival of dormant mold spores on the corn after IR and tempering treatments. When placed in regrowth conditions, these dormant mold spores begin to germinate again. This is further corroborated by Dettori et al. (1996) who found that hot water dips at 50-53°C was proven to be ineffective in killing dormant spores. However, the hot water treatments did cause a delay in conidia germination, growth and sporulation.

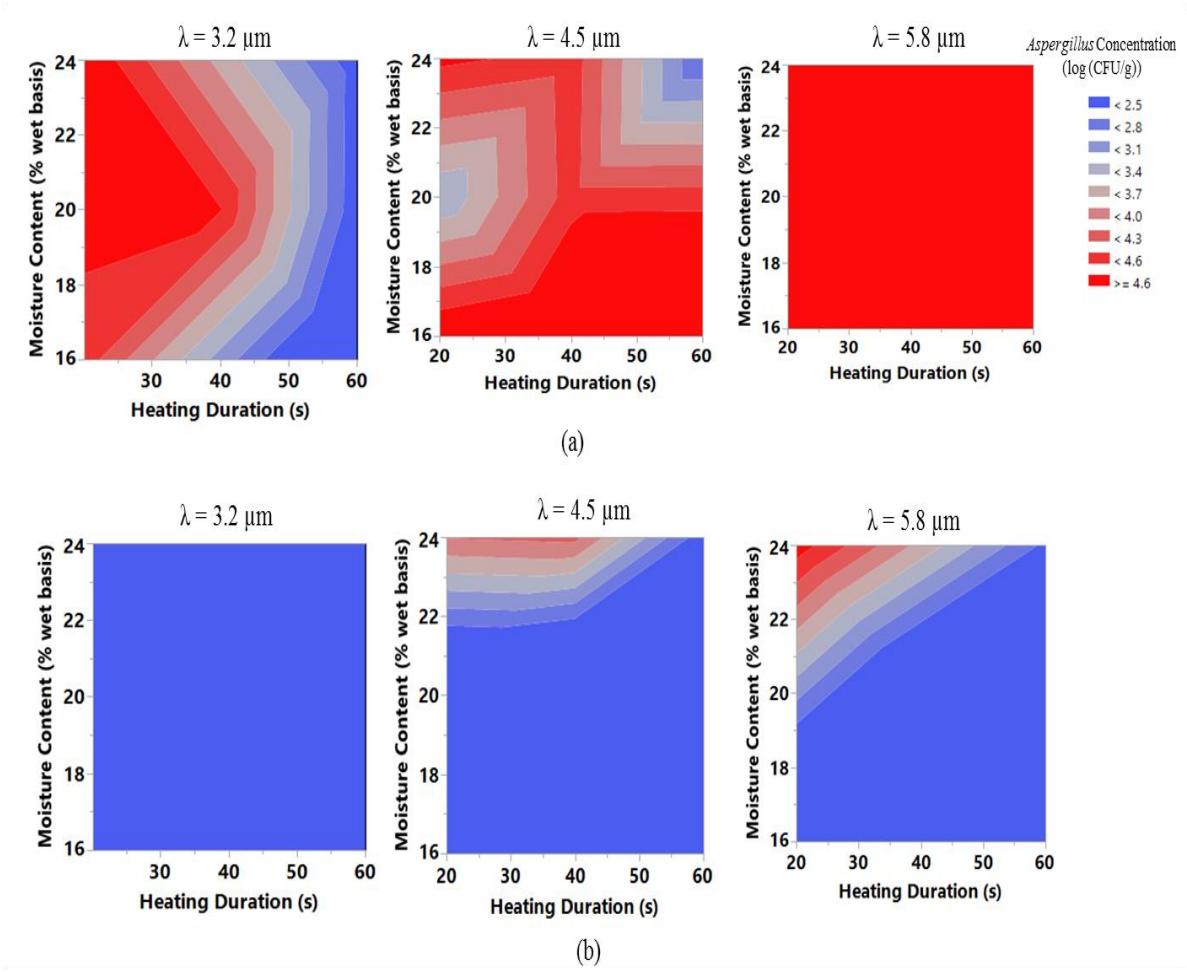

Fig. 4. Contour plots of mold counts (log CFU/g) as influenced by the interaction between heating duration (s), wavelength (µm) and corn moisture content (% w.b.) for non-tempered (a) and tempered treatments (b), respectively; CFU means colony forming units; λ signifies wavelength in µm.

After a RSM was conducted, it was determined that there was a significant difference in the *A. flavus* concentration based on the effects of PEG, heating duration, MC, and wavelength for non-tempered and tempered treatments (Table 2 and 3). Table 6 shows the effect summary table for the mold count response. Smaller p-values indicate higher significance to the model. For non-tempered samples, the main effect of wavelength (0.02204) and interaction between wavelength and heating duration (0.03773) was statistically significant. Although, the main effect of heating duration was not significant, it was kept in the model because the interaction

with wavelength was significant. For tempered samples only, the main and quadratic effect of moisture content was significant (Table 4).

Table 2. Effect test table showing the effects of selective infrared heat treatment on the *Aspergillus flavus* for non-tempered samples.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	2	13.302130	6.65107	8.5425	0.0025*
Error	18	14.014467	0.77858		
C. Total	20	27.316597			

Table 3. Effect test table showing the effects of selective infrared heat treatment on the *Aspergillus flavus* for tempered samples.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	3	14.090253	4.69675	5.8709	0.0061*
Error	17	13.600151	0.80001		
C. Total	20	27.316597			

Table 4. Effect summary table showing the effects of wavelength, heating duration and moisture content on the *Aspergillus flavus* concentration (Log (CFU/g-grain).

Source	P Values	
	Non-Tempered	**Tempered**
Wavelength (μm)(3.2,5.8)	0.02204	> 0.05
Wavelength (μm)*Heating Duration (s)	0.03773	> 0.05
Heating Duration (s)(20,60)	0.08001	> 0.05
Moisture Content (% w.b.)(16,24)	> 0.05	0.00428
Moisture Content (% w.b.)*Moisture Content (% w.b.)	> 0.05	0.02088

Conclusion

This study examined the use of IR treatments followed by tempering to decontaminate corn and limit the regeneration of *A. flavus* spores on the grain. The *A. flavus* concentration of the control sample was significantly higher than the *A. flavus* concentration of samples treated with selective IR. It was also noted that increasing IR intensity and treatment durations supplied to the corn resulted in decreasing *A. flavus* mold concentrations. The optimal condition for reduction in *A. flavus* concentration was at wavelength of 3.2 μm (intensity of 15.71 kW/m^2), corn MC of 24% w.b. and PEG of 110 mm. The addition of a tempering step resulted in a significant reduction in *A. flavus* concentration. Corn with lower MC showed less potential for *A. flavus* regrowth after 5 days of incubation in favorable conditions for mold growth (33^o C, and 90% RH). The treatments studied provide an avenue for implementing a non-chemical approach to corn decontamination. If optimized, the technology can help prevent mold-related hazards and improve corn quality.

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Chapter 6: Conclusions

This study evaluated the impact of exposing corn to infrared energy at selected peak wavelengths (λ), infrared intensities and treatment durations, followed by tempering for further inactivation of microbes on the grain. The study also examined the potential to limit the regeneration of *A. flavus* spores on the grain. The selective infrared treatment reduced the microbial load on the corn. There was a decrease in microbial load with an increase in MC and heating duration. The lower wavelength and product-to-emitter gap the greater the reduction in mold count. It was also noted that increasing IR intensity and treatment durations supplied to the corn resulted in decreasing *A. flavus* mold concentrations. After 5 days of incubation, corn with lower MC showed less potential for *A. flavus* development under favorable circumstances for mold growth

Optimization analyses suggest that a wavelength of 3.2 μm (intensity of 15.71 kW/m^2), a heating duration of 60 s, corn MC of 24% w.b., and a PEG of 110 mm provide the optimal response in terms of corn mold load reduction and *A. flavus* concentration. Tempering created a synergistic effect which allowed for further reduction in microbial load population and *A. flavus* concentration on corn.

Metagenomics analysis found that Ascomycota was the most dominant phylum for non-tempered and tempered treatments. After the IR treatments it was noted that the fungal community was separated by the different corn MCs.

In summary, selective IR treatment approach as opposed to broadband IR treatment could be effective in inactivating molds, especially those responsible for mycotoxin production on shelled corn. When fully optimized, the newly developed selective IR heating process has

potential to maximize inactivation of mycotoxigenic fungi and contribute significantly to knowledge and science.