DETOXIFICATION OF AFLATOXIN IN CONTAMINATED PEANUTS BY ULTRAVIOLET RADIATION, HYDROGEN PEROXIDE, AND THEIR COMBINATION

by

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(Under the Direction of Rakesh K. Singh)

ABSTRACT

Aflatoxin contamination is a serious problem in the global food supply chain. This study was aimed at investigating the use of ultraviolet radiation (UV), hydrogen peroxide (H_2O_2), and their combination to detoxify aflatoxin in contaminated peanuts, as well as the quality changes in peanut kernels after treatments. To analyze aflatoxin concentration changes, a method employing immunoaffinity column and normal phase HPLC-FLD was developed. The method had better performance and less time and preparative procedures than that of the official method. To determine the uniformity of UV radiation, peanuts were coated by AgCl as the UV dosimeter and rotated in a drum at different speeds. The dose of radiation received on each area of peanuts was measured by color change after UV treatment. Rotating the drum at 11 rpm uniformly distributed UV radiation on peanuts and increased aflatoxin reduction by 23%. To evaluate the effects of different wavelengths on aflatoxin reduction and oil quality of peanuts, three low-pressure UV lamps emitting UV-A, UV-B, and UV-C were used to decompose aflatoxin. The low-pressure UV-A lamp demonstrated a higher aflatoxin reduction (26%) in an hour of irradiation than that of UV-B (16%) or UV-C (21%) and had a lower effect on oil quality of aflatoxin-spiked peanuts than those treated by UV-C. To understand the interaction between the contaminated peanuts and H_2O_2,

peanuts were subjected to short time roasting to inactivate the catalase followed by the treatment with 30 g/hg of H_2O_2 at 50˚C. A 90% aflatoxin reduction was observed in aflatoxin-spiked peanuts after being subjected to the treatment for 8 h, but the oil quality was not seriously affected. The aflatoxin reduction found in peanuts was higher than in the model solution, indicating that peanuts may assist in aflatoxin degradation. To further improve the aflatoxin reduction efficiency, low-pressure UV-C lamps and H_2O_2 were combined to decompose aflatoxin in spiked peanuts. The treatment reduced 33% of aflatoxin in an hour. The oil quality was slightly affected. The residual H_2O_2 was completely removed by drying treated peanut at 35˚C for 12 h. These results indicate that the combination treatment significantly increased aflatoxin reduction efficiency.

INDEX WORDS: Non-thermal processing, Radiation uniformity, Normal-phase HPLC, UV wavelength, Hydrogen peroxide, Advanced oxidation processes

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DEDICATION

This dissertation dedicates to my parents, teachers, and advisors who inspired me to start my academic career, and to those people who have made an effort against hunger.

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CHAPTER 1

INTRODUCTION

Aflatoxin (AF) is a type of mycotoxin of the most concern due to its adverse health effects on mammals and prevalence in our food supply (FAO, 2017; Scott, 1984). Consuming AF-contaminated foods can lead to failure of the human liver and kidney, as well as the impairment of the immune system and the growth of children (Chen et al., 2018; Mousavi Khaneghah et al., 2018). AF has been recognized as the class 1 carcinogen by IARC (2022). FAO (2002) estimated that more than 25% of worldwide crops might be contaminated with AF. Crops contaminated with excessive AF levels are not for animal feed since AF metabolites can be detected in animal products when feeding the animals with AF-contaminated crops (Atherstone et al., 2016). Authorities around the world have set different activity levels to regulate the concentration of AF in not only human foods but also animal feeds.

Several physical and chemical methods have been proposed to reduce AF in contaminated commodities. However, drastic conditions such as high temperature or alkali were introduced in a detoxification processing might cause considerable deterioration of quality in treated foods or limit the use of those foods for a variety of purposes (Samarajeewa et al., 1990; Tabata et al., 1994). Accordingly, this research project was aimed at reducing AF in contaminated foods with more efficient methods but not considerably affecting the quality of foods or having serious safety concerns to humans. To reach that goal, we investigated the use of UV radiation, hydrogen peroxide, and their combination treatment. Peanut was used as the material in this study due to its vulnerability to being infected by *Aspergillus*, which contributes to the most of AF contamination.

UV radiation can reduce AF and degrade them into less toxic compounds with almost no effect on oil quality (Diao et al., 2015; Stanley et al., 2020). The treatment can be considered as a "non-thermal processing." Also, the radiation has no residue issues which may lead to health concerns. However, the detoxification efficiency of UV is usually low. Many studies made a speculation that the efficiency could be higher if irradiating UV on the sample surface uniformly, but until now no research has been conducted to confirm that aspect (Altuğ et al., 1990; Jubeen et al., 2012). Additionally, comprehensive knowledge regarding the effect of different UV wavelengths on AF degradation and food quality is still scarce.

H_2O_2 has been approved as a food ingredient or sanitizer in the food processing industry. The residual H_2O_2 can be easily removed by conventional unit operations such as drying. However, high temperature or alkaline condition is usually needed to catalyze the oxidation process, especially when the H_2O_2 concentration is as low as about 1 g/hg, which was usually applied in previous studies. These conditions might considerably impair the quality of the treated foods (Samarajeewa et al., 1990; Tabata et al., 1994). Additionally, H_2O_2 concentration may significantly decrease due to catalysis by enzymes in most foods. Maintaining the concentration of H_2O_2 during a detoxification process may therefore increase the AF reduction.

Introducing UV to generate hydroxyl radical (HO•) and hydroperoxyl (HOO•) from hydrogen peroxide, or "advanced oxidation processes," is another potential way to promote AF decomposition (Glaze et al., 1987). Additionally, these radicals can diffuse into the area where UV may not reach, further assisting in AF decomposition. The reaction can spontaneously occur in a short time, so no additional catalyst or thermal processing is needed, which should help preserve most nutrients in peanuts.

These findings lead to our **central hypothesis** that the AF detoxification processes mentioned above are inefficient since the processing conditions are not appropriately set up. Specifically, we envisioned that (1) uniformly irradiating peanuts with UV radiation can increase AF reduction, (2) AF reduction rate and quality of peanuts can be affected by UV radiation at different wavelength ranges, (3) treating enzyme inactivated peanuts with a high concentration of H_2O_2 can accelerate AF degradation, and (4) using a combination treatment of UV and H_2O_2 can reduce AF more efficiently. The **overall purpose** of this study was to determine parameters that can improve the AF detoxification efficiency of UV or H_2O_2 treatments and can be practically applied by the food industry or the public. To examine the hypothesis, four specifics objectives were proposed:

(1) To develop a radiation uniformity quantification method and investigate the influence of radiation uniformity on AF reduction in whole peanut kernels.

(2) To evaluate the effect of different wavelength ranges on AF reduction and oil quality change of peanuts.

(3) To determine the effects of using high concentration H_2O_2 and inactivating H_2O_2 catalyzing enzyme in peanuts on AF reduction.

(4) To investigate the AF reduction efficiency and the oil quality changes of UV/ H_2O_2 combination treatment in peanuts.

In this dissertation, a review of literature is in Chapter 2. To reduce the laborious and time-consuming experimental protocols for AF analysis, a modified HPLC method and its detailed description are in Chapter 3. The four proposed objectives were then performed and are covered in Chapter 4 through Chapter 7. A comprehensive conclusion and future recommendations of the entire dissertation are in Chapter 8.

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CHAPTER 2

DETOXIFICATION OF AFLATOXINS IN FOODS BY ULTRAVIOLET IRRADIATION, HYDROGEN PEROXIDE, AND THEIR COMBINATION - A REVIEW[1]

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Abstract

Aflatoxins (AFs) contamination is prevalent in foods around the world. Many studies have been performed to recover foods contaminated with unacceptable levels of AFs. This review evaluated AFs detoxification technologies of ultraviolet (UV), hydrogen peroxide (H_2O_2), and their combination. Microscopic evidence has demonstrated that AFs are mainly distributed on or near the surface of contaminated foods, and thus a treatment applied near the surface would be sufficient for detoxification. UV is an ideal treatment due to its ability to detoxify AFs, affordable cost, and less quality impact. UV can degrade aflatoxin B1 into more than 200 times less toxic compound, aflatoxin B2a, justifying the use of this method in practice. However, the "shadow effect" and poor penetrability are concerns when applying UV on solid materials. H_2O_2 can oxidize AFs into less toxic compounds with negligible quality change and no residual issue after treatment. H_2O_2 is usually combined with heat, radiation, or alkali to promote the oxidation process. Combining UV and H_2O_2 (Advanced Oxidation Processes, AOPs) can photolyze H_2O_2 into free radicals, which can oxidize most organic compounds and has been used for pollutants decontamination in water treatment. Previous works demonstrated that AOPs could successfully detoxify AFs in contaminated food.

Introduction

Aflatoxins (AFs) are the mycotoxins of most interest due to their prevalence in foods around the world as well as a cause of adverse health effects to mammals, such as mutagenic and carcinogenic potential (JECFA, 2017). AFs are secondary metabolites mainly produced by *Aspergillus* spp. belong to section *Flavi* such as *Aspergillus flavus* or *Aspergillus parasiticus* (Klich, 2007; Mousavi Khaneghah, Ismail, Raeisi, & Fakhri, 2018). There are five common types of aflatoxin: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), and aflatoxin M1 (AFM1). International Agency of Research on Cancer has recognized these five common toxins as group 1 carcinogens (IARC, 2020). AFM1 is a metabolite of AFB1 produced in the liver, being excreted mainly into the milk of lactating animals (Campagnollo et al., 2016; JECFA, 2001).

The permissible AFs level is regulated by the authorities for preventing the entry of unacceptable contamination into the food supply chain. The AFs levels of 20 μg/kg for human foods and 100-300 μg/kg for different animal feeds are permitted in the U.S. Based on the U.S. Food and Drug Administration (FDA) policy, corns contaminated with actionable levels of AFs after an attempt at reconditioning should not be used as human foods and animal feeds (U.S. FDA, 2000). The AFs contamination was estimated to cause $ 0.05 to $ 1.68 billion of losses to the corn industry in the U.S. annually (Mitchell, Bowers, Hurburgh, & Wu, 2016). Several studies are proposed to physically or chemically recover heavily contaminated foods by reducing AFs to the acceptable level. Biological detoxification processes such fermentation largely change the form of the foods and will not be discussed in this paper.

Physical methods mainly use segregation, thermal process, and radiation to lower the AFs concentration. A segregation process can be simply completed by sorting, absorbing, or washing

out the AFs from foods (Karlovsky et al., 2016). Sorting could be significant but incomplete since the presence of fungi growing can be only recognized when severe contamination occurred (Karlovsky et al., 2016). The recovery of absorbent and solvent used for absorbing or washing would be an issue (Piva, Galvano, Pietri, & Piva, 1995). A thermal process has been considered inefficient, and it would change the properties of the product. Although roasting (200°C, 25 min) was able to reduce 90% of AFs in contaminated peanuts, the low L value of 39.3 indicated that the peanuts were nearly scorched and presented a dark color (Martins et al., 2017). Radiation methods usually refer to the ionizing or partially ionizing treatments by electromagnetic spectrum with short wavelengths, such as gamma irradiation or UV irradiation. With a sufficient dosage of gamma irradiation, a notable detoxification rate and almost no quality change were reported (Ghanem, Orfi, & Shamma, 2008; Patel, Govindarajan, & Dave, 1989). However, the cost of gamma irradiation equipment and consumer hesitation about irradiated foods is a concern. UV irradiation is a disinfection process that uses radiation with a wavelength from 100 to 400 nm to food subjects (Guerrero-Beltrán & Barbosa-Cánovas, 2004). UV irradiation has a similar effect as gamma irradiation on decontamination AFs, but the "shadow effect" and its low penetrability limit the application of UV in solid foods (Shen & Singh, 2021). The other concern for employing UV is the processing time usually needs several hours to complete. This review provides a potential solution for mitigating these issues.

Chemical methods for detoxification are of interest due to their profound efficiency and affordable cost. The primary purpose of chemical treatment is to activate AFs molecules by oxidation, hydrolysis, or addition reaction, rendering AFs to be degraded. The addition of acids or oxidants triggers the oxidation at the C8 position to form a hemiacetal, which is less toxic and easy to be hydrolyzed (Tabata, Kamimura, Ibe, Hashimoto, & Tamura, 1994). Nonetheless, the process

was time-consuming and not suitable for human foods. Alkaline treatment can hydrolyze the lactone ring inside AFs molecules, but the process is reversible in acidic conditions, which might maintain the toxicity of AFs (Figure 2.1) (Price & Jorgensen, 1985). Ozonation can significantly reduce AFs levels, but the whole process may take several hours to complete (Porto et al., 2019). Besides, ozone is toxic and explosive. The equipment to eliminate these safety concerns might not be affordable to most food suppliers. Ammoniation can open the lactone ring similar to alkaline treatment, and its detoxification ability has been confirmed by the U.S. Department of Agriculture (USDA) (Samarajeewa, Sen, Cohen, & Wei, 1990). However, significant losses of lysine and methionine were reported after ammoniation. Reactive agents such as sodium hypochlorite or sodium bisulfite can undergo chlorination or sulfonation at the C8 or C9 positions, activating AFs molecules to be decomposed (Samarajeewa et al., 1990). Although these chemical methods can effectively detoxify AFs, the safety of the degraded compounds and the removal of residual chemicals after treatments largely limit their application.

The microscopic evidence reveals that drastic physical or chemical treatments might not be necessary for detoxification. Scanning electron microscope (SEM) images showed that the hyphae of *Aspergillus* spp. predominantly grown on or near the oxygen-available surface of peanuts (Achar, Hermetz, Rao, Apkarian, & Taylor, 2009). A considerable AFs reduction was found in the corn dehulling process, indicating that AFs are mainly distributed on the hull of corn kernels (Siwela, Siwela, Matindi, Dube, & Nziramasanga, 2005). The sliced sections of contaminated peanuts showed that the AFs were mainly distributed near the surface or at the germ of peanuts (Cucullu, Lee, Mayne, & Goldblatt, 1966). These observations justified the use of UV irradiation as a surface treatment to detoxify AFs. Hydrogen peroxide, which can be easily removed after the detoxification treatment, is a strong oxidant for oxidating AFs, especially when

irradiated with UV. Irradiating UV on hydrogen peroxide can generate much more free radicals than hydrogen peroxide alone. The process is also known as “advanced oxidation processes” (AOPs) and is widely used in oxidating organic compounds. This paper reviews the studies using UV and hydrogen peroxide and discusses the potential of combining two treatments to detoxify AFs in contaminated food.

Decontamination of aflatoxins by UV

Studies focusing on detoxifying AFs by UV are still emerging due to the affordable expense of equipment, negligible impact on food quality, and less residual toxicity issues of the UV-processed products. This review includes, but is not limited to, the recent works critical on this area (Table 2.1).

Recently, researchers have paid more attention to studying food quality change and improving the detoxification efficiency in a UV detoxification process. Tripathi & Mishra (2010) concluded that using 12 units of peroxidase followed by a 30-minute UV irradiation degraded 77% of AFB1 in contaminated chili powder without considerable changes of ascorbic acid, total carotenoids, and total capsaicin. The additional UV treatment significantly increased aflatoxin reduction and decreased revertant rates in an Ames mutagenicity test.

Garg et al. (2013) achieved a 99% reduction of AFs in contaminated peanut without significant deterioration of the oil extracted from treated peanuts. The only noticeable influence on the quality of oil after 12-hour of UV exposure was slightly raised acid value (AV) from 1.39 mg KOH/g of oil to 2.46 mg KOH/g of oil. The change in peroxide value (PV) and saponification value (SV) were negligible. A 6-log reduction of fungal contamination was also observed. However, the time needed to complete a detoxification process may restrict its practical applications.

Decontaminating AFs in contaminated peanut oil by UV was also investigated (Magzoub et al., 2019). A photocatalyst, TiO_2, was introduced to accelerate the AFs degradation. The results demonstrated that UV irradiation of peanut oil previously mixed with TiO_2 particles shortened the detoxification process from 6 min to 4 min. The enhanced degradation rate could be contributed to the oxidation of AFs by free radicals such as superoxide or hydroperoxyl radicals produced by UV and photocatalyst. After UV exposure, the AV, PV, and SV of the peanut oil were almost identical. The iodine value was significantly increased but acceptable. The nutritional values of UV-treated foods should not be considerably impaired due to the low penetrability of the radiation, which hardly affects the nutrient far from the surface of treated foods.

Ozone was introduced and coupled with UV irradiation to enhance AFs degradation rate (Li et al., 2019). The authors concluded that contaminated peanuts treated with 5 mg/L ozone under UV irradiation for 30 min could degrade 79% of AFs without significant changes of polyphenols, AV, and PV. The idea of providing additional oxidants to accelerate AFs degradation under UV irradiation has therefore been approved.

However, the “shadow effect” occurred when the solid foods were irradiated with UV, and the concept of increasing AFs reduction by evenly exposing the sample surface was investigated (Shen & Singh, 2021). Because of the low penetrability, UV can hardly penetrate solid foods and the shaded areas get blocked from receiving the irradiation. An uneven UV irradiation distribution due to the irregular shape of the foods or the varied distance between samples and UV sources may cause incomplete disinfection of AFs. After evenly irradiating the whole peanut surfaces by UV, a higher AFB1 reduction was observed by Shen & Singh (2021). This result confirmed the hypothesis that the AFs reduction can be further improved by evening UV radiation on the AFs

contaminated foods (Altuğ, Yousef, & Marth, 1990). That is, how to evenly irradiate UV on whole surface of treated food would be an issue, especially when processing foods with irregular shapes.

UV treatment has been proved to degrade AFs to less toxic compounds. For example, AFB2a (hydroxy AFB1) is formed from AFB1 by free radical attack under UV irradiation. A hydrogen atom at the C8 position is oxidized to a hydroxyl group (Figure 2.1). The process has been applied as a post-column derivative method to increase the fluorescence detectability in liquid chromatography (LC) analysis (Joshua, 1993). AFB2a was estimated to be more than 200 times less toxic than AFB1 in the duckling feeding test (Lillehoj & Ciegler, 1969). Under certain conditions such as acidic treatment, AFB2a, as a hemiacetal, can reversibly convert to AFB1 dialdehyde, which is considered as a potential cytotoxic compound in metabolic pathways (Figure 2.1) (Johnson, Harris, & Guengerich, 1996). However, AFB1 dialdehyde could presumably react with amino groups within the food matrix to form a Schiff base, eventually lowering its bioavailability. This may explain the less toxicity of AFB2a observed in duckling feeding tests. After UV treatment, lower cytotoxicity of AFs degradation products was reported (Patras et al., 2017). In their study, human hepatocytic HepG2 cells were not significantly affected by treating with AFs-water solution previously exposed to 4.88 J/cm^2 of UV.

Although these results demonstrate that UV treatment was an effective AFs detoxification process, more studies are needed to refine the method. Combining other AFs detoxification techniques to promote the detoxification process and lower the quality impact is necessary. Further, even irradiation and eliminating the "shadow effect" are the direction to improve incomplete disinfection. The effect of residual toxicity on humans should also be studied even though the preliminary degradation compounds from AFs have been proved less toxic. Moreover, the effects of different UV lamp types on the detoxification process and food quality have not been well

studied. Three major types of lamps, low-pressure, medium-pressure, and high-pressure lamps, are usually used. Low-pressure lamps emit a low-power UV (several milliwatts per cm^2) within a narrow wavelength band. Medium-pressure lamps emit an intense (hundreds of W per cm^2) UV within a broader continuous wavelength band across the whole UV band. High-pressure lamps emit an intense UV (hundreds of watts per cm^2) but within a narrow wavelength band. The intense radiation from medium- and high-pressure lamps may seriously impair the quality of foods when detoxifying AFs. Also, the expense for equipping medium- and high-pressure lamps is much higher than low-pressure lamps because of the shorter life of the lamps as well as the kilowatt power supply for driving the lamps.

Detoxify aflatoxins by H_2O_2

Compared to the research applying UV irradiation, studies focusing on degrading AFs by hydrogen peroxide have been of less interest, especially after the year 2000. Although some of the studies reviewed in this paper were published more than 20 years ago, they are very relevant and contain valuable information to be discussed because of their contributions to AFs detoxification (Table 2.2).

Hydrogen peroxide was considered as a potential treatment that can effectively detoxify AFs due to its oxidation ability when combined with other detoxification methods. A 97% of AFs reduction in defatted peanut meal was reported by treating the meal with alkalized 6 g/hg hydrogen peroxide solution at 80°C for 30 min (Sreenivasamurthy, Parpia, Srikanta, & Murti, 1967). The duckling tests indicated that the toxicity was almost completely reduced, as evidenced by negligible liver lesions and changes in body weight gain of ducks fed with treated meals. The protein efficiency ratio of treated peanut meals was not significantly different from those without treatment. Also, the authors reported that no fluorescent response was found in AFs degradation

compounds in thin layer chromatography analyses, which revealed that the main structures of AFs were destroyed. The future works should focus on the effect of H_2O_2 on amino acid quality.

The synergistic effect of hydrogen peroxide and gamma irradiation was investigated (Patel et al., 1989). A 77% reduction of AFB1 was reported after suspending contaminated peanuts in 5 g/dL of hydrogen peroxide and irradiating with 2 kGy of gamma irradiation. The degradation products after the treatment did not cause mutagenicity in the Ames test. The authors concluded that hydrogen peroxide needed to be associated with gamma irradiation for shortening the detoxification time. They hypothesized that more free radicals would be disassociated from hydrogen peroxide under gamma irradiation, resulting in a higher degradation rate of AFB1.

Altuğ et al. (1990) proposed combining hydrogen peroxide and sodium bisulfite to detoxify AFB1 contaminated dried figs. Treating 20 g contaminated dried figs with 20 g of 1 g/hg sodium bisulfite alone at 25°C reduced 28% of AFB1 within 72 h, whereas treating 20 g of dried figs firstly with 10 g 0.4 g/hg hydrogen peroxide for 10 min followed by 10 g of 2 g/hg sodium bisulfite considerably increased the AFB1 reduction rate to 68%. The authors also hypothesized that the addition of sodium bisulfite could minimize the oxidation damage in figs caused by hydrogen peroxide.

The effect of hydrogen peroxide on AFs detoxification was compared with other typical food additives such as $Na_2S_2O_4$, $NaHSO_3$, $NaClO_2$ (Jalili, Jinap, & Son, 2011; Tabata et al., 1994). Tabata et al. found that mixing contaminated corn grit with 1 g/dL of hydrogen peroxide at 60°C for 48 h was able to degrade 68% of AFB1, 86% of AFB2, 100% of AFG1, and 100% of AFG2. However, the detoxification efficiency of hydrogen peroxide was much lower than that of sodium chlorite, which completely degraded AFs in 16 h, but the residual toxicity and the quality impairment after sodium chlorite treatment were questionable. The authors hypothesized that the

observed low detoxification efficiency could be due to the loss of hydrogen peroxide reacted with foods or the protection of AFs by food components such as starch or reducing saccharides. On the other hand, Jalili et al. (2011) reported that 2 g/dL of hydrogen peroxide effectively reduced 44% of AFB1 in black pepper and 45% of AFB1 in white pepper at room temperature within 2 hours. The addition of hydrogen peroxide bleached the color of the white pepper but damaged the skin of the black pepper.

Elias-Orozco et al. (2002) indicated that using a single-screw extruder (87°C, 35 rpm) to process cornflour mixed with 3 g/hg of hydrogen peroxide reduced 72% of AFB1, whereas extruding cornflour mixed with 3 g/hg of hydrogen peroxide and 0.3 g/hg of calcium hydroxide reduced 78% of AFB1. However, the overuse of chemical treatment will cause off-flavor and taste in the final product, compared to the tortillas made by the traditional nixtamalization process.

The mechanism of degrading AFs by hydrogen peroxide is not yet clear. Hydrogen peroxide is recognized as a powerful oxidizer. Patel et al. (1989) described the unknown degraded compounds were found to have a strong fluorescence response. This finding implied that hydrogen peroxide might oxidize the hydrogen atoms located at the C8 position of AFB1 to form AFB2a in the initial degradation stage, similar to the derivatization process in an LC analysis.

Hydrogen peroxide treatment may have an impact on sensory attributes and nutrition values of treated foods, but the total influence could be negligible. Bayoumi (2008) found that treating whole green pepper fruits with 15 mmol/L of hydrogen peroxide for 2 weeks can significantly reduce rot rate but increase the general appearance and ascorbic acid content. On the other hand, Kim, Fonseca, Kubota, & Choi (2007) documented that dipping fresh-cut tomato in 0.4 mol/L of hydrogen peroxide for 1 min can inhibit microbial population enhancement after 8 days, but the total phenolic, ascorbic acid, and lycopene content decreased about 5-20%. The color

of the treated tomato also became lighter. The change could be due to the lower content of lycopene. In practice, the use of hydrogen peroxide for AFs detoxification should be controlled to occur near the surface of foods to minimize the influence on sensory attributes and nutrition values.

Using hydrogen peroxide alone to degrade AFs is inefficient. Tabata et al. concluded that hydrogen peroxide is an ideal additive to degrade AFs because hydrogen peroxide can be easily removed after treatment, but a method for accelerating the oxidation process is needed (Tabata et al., 1994). The alkaline condition enhanced the AFs reduction by hydrogen peroxide, but the increase may not result from the promotion of oxidation (Elias-Orozco et al., 2002; Sreenivasamurthy et al., 1967). One possible explanation is that under alkaline conditions, the lactone ring in AFs may be hydrolyzed and opened. The open-ring compounds are not detectable by traditional UV detectors, and therefore the actual AFs concentrations were underestimated (Price & Jorgensen, 1985). The confirmed method to accelerate the oxidation process is to disassociate hydrogen peroxide to hydroxyl radicals using ionization methods such as gamma irradiation or UV (Patel et al., 1989). Hydroxyl radicals are highly reactive molecules. The process, as known as "advanced oxidation processes" (AOPs), theoretically oxidizes most of the organic compounds into carbon dioxide and water (Glaze, Kang, & Chapin, 1987). Another method to increase the oxidation rate of hydrogen peroxide is a thermal process, but the quality and nutrients of subjected foods may be largely lost during cooking or boiling (Tabata et al., 1994). The future work of applying hydrogen peroxide should focus on promoting the oxidation process while maintaining an acceptable quality of foods.

Detoxification of aflatoxins by UV coupled with H_2O_2

The techniques applying a high concentration of free radicals to disinfect contaminations such as AOPs have been introduced in the water treatment system, yet only a few researchers using

the techniques in food disinfection, especially in the AFs detoxification area. Gamma irradiation has a similar effect on promoting free radicals generated from hydrogen peroxide, but the high expense for the equipment limits its applications. UV is a more affordable option and thus is widely used. Additionally, it is much easier to customize the combined treatment by UV and hydrogen peroxide for different situations. Recently, AOPs regains focus due to the ability to disinfect pathogens or contaminants efficiently. We will discuss studies aimed at disinfecting pathogens by AOPs and justify its use in detoxifying AFs.

AOPs were reported to detoxify AFM1 in milk effectively and potentially detoxify AFs in other foods. An 89% reduction of AFM1 in contaminated milk was reported after the addition of 0.05 g/dL hydrogen peroxide and a 20-minute UV irradiation, whereas UV exposure alone reduced 61% of AFM1 (Yousef & Marth, 1986). The milk was heated to 90°C and held for 10 min to eliminate the influences from enzymes in the milk. Although the AFs reduction was significant, the combined treatment effect on the quality of milk was not discussed in detail. The combined treatment was also examined in detoxifying AFs contaminated dried figs, but the results were not significant, compared to UV only (Altuğ et al., 1990). This result could be due to the limited area of samples exposed to UV. The addition of sodium sulfite could react with the generated free radicals and "protect" the oxidation of AFs.

On the other hand, effectively disinfecting pathogens in contaminated foods by AOPs can justify using combined treatment in detoxifying AFs. A 30-second UV irradiation (37.8 mJ) coupled with 1.5 mL/dL of 50°C hydrogen peroxide reduced *Salmonella* on the surface and within the subsurface of lettuce by 4.12 and 2.84 log CFU per 25 g sample, respectively (Hadjok, Mittal, & Warriner, 2008). Compared to UV or hydrogen peroxide only, the significantly higher disinfection rate suggested the lethal effect would primarily come from the combined treatment.

Further, the increase of the *Salmonella* reduction inside the lettuce implied that the generated free radicals were able to diffuse into the area where UV cannot arrive due to the "shadow effect" or poor penetrability. Moreover, the authors reported that the shelf-life stability of the treated produce was not significantly affected by the treatment.

The study employing the combination method to detoxify AFs in solid contaminated foods such as grains has not yet been documented. However, we can envision that the method has the potential to mitigate the issue. First, AFs are soluble in water, indicating that AFs will dissolve from contaminated foods to the hydrogen peroxide solution (Karlovsky et al., 2016). Second, AOPs can decompose most organic compounds, even aromatic halides, and therefore should be able to decompose AFs dissolved in hydrogen peroxide solution (Glaze et al., 1987; Miklos et al., 2018). Even though the detoxification process by the AOPs method could also affect the quality of foods, the extent of quality impairment should be acceptable because the AOPs will be controlled to occur near the surface of foods where the AFs dominantly distribute.

Conclusions

The reviewed studies demonstrated that the combination of UV and hydrogen peroxide is a potential method to detoxify AFs contaminated foods within a shorter period than by other methods. The combination can mitigate the shortcomings of each treatment alone. The generated free radicals enhance the AFs degradation rate, considerably shortening the time needed for oxidizing AFs when using hydrogen peroxide alone. Additionally, the diffusive ability of generated free radicals helps decompose AFs in the deeper internal area inside the foods, mitigating the "shadow effect" and the low penetrability of UV. Furthermore, the residual hydrogen peroxide can be simply removed by a thermal process or rinse after the detoxification process. Also, the entire process can be considered a non-thermal process.

However, as an emerging technique, using AOPs for AFs detoxification needs more research on the safety and quality of treated foods. Even though the theoretical degradation compounds should be less toxic or even non-toxic, the residual toxicity after treatment still needs more investigation. The impact of the treatment on sensory attributes and nutrient values should also be evaluated as these free radicals vigorously react with most of the organic compounds found in foods.

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Tables

Table 2.1 Detoxifying AFs by UV irradiation.

Treatment	Subject	AFs Reduction (%)	Reference
365nm UV lamp with garlic peroxidase, 30 cm above the subject, 60 min	Red chili powder	77% of AFB1	Tripathi & Mishra (2010)
15W UVC lamp, 1.5 mW/cm^2, 12 h	Whole peanut kernel	99% of AFs[a]	Garg et al. (2013)
1000W UV lamp, 4.88J/cm^2	AFs in water solution	98% of AFB1 30% of AFB2 67% of AFG1	Patras et al. (2017)
500 W high-pressure lamp with TiO_2, 200 mW/cm^2, 4 min	Peanut oil	≥99.4% of AFB1 ≥99.2% of AFB2	Magzoub et al. (2019)
254 nm UV lamp with 5 mg/L of ozone, 0.35mW/cm^2, 30 min	Whole peanut kernel	79% of AFB1 67% of AFB1+AFB2+AFG1+AFG2	Li et al. (2019)
15W UVC lamp, 2.3 mW/cm^2, 2 h	Whole peanut kernel	18% of AFB1	Shen & Singh (2021)

[a] The authors did not describe which types of AFs.

Table 2.2 Detoxifying AFs by hydrogen peroxide.

Treatment	Subject	AFs Reduction (%)	Reference
5 g of meal, 5 mL of 6 g/hg H_2O_2 at pH 9.5, 50°C, 30 min[a]	Defatted peanut meal	97% of AFs[b]	Sreenivasamurthy et al. (1967)
10 g of peanuts, 25 mL of 5 g/dL H_2O_2 with 2 kGy of gamma irradiation, 30 min	Unshelled peanut	77% of AFB1	Patel et al. (1989)
20 g of figs, 10 g of 0.4 g/hg H_2O_2 followed by 10 g of 2 g/hg $NaHSO_3$, 72 h[a]	Dried fig	68% of AFB1	Altuğ et al. (1990)
1 g of grit, 10 mL of 1 g/dL H_2O_2, 60°C, 48 h[a]	Corn grit	68% of AFB1 86% of AFB2 100% of AFG1 100% of AFG2	Tabata et al. (1994)
100 g of pepper, 2 g/dL H_2O_2, room temperature, 2 h[a]	Black pepper[c]	44% of AFB1 32% of AFB2 43% of AFG1 37% of AFG2	Jalili et al. (2011)
	White pepper[c]	45% of AFB1 35% of AFB2 40% of AFG1 35% of AFG2	
3 g/hg H_2O_2 with 0.3 g/hg $Ca(OH)_2$, extrusion at 87°C at 35 rpm	Corn flour[d]	78% of AFB1 81% of AFM1 84% of AFB1-8,9-dihydrodiol	Elias-Orozco et al. (2002)

[a] The specific concentrations of the additives were not given. The values shown in this table are estimated according to the context in each study.

[b] The authors did not describe which types of AFs.
[c] Hydrogen peroxide treatment bleached the color of the white pepper but damaged the skin of the black pepper.
[d] Overuse of the chemical treatment will cause off-flavor and taste in the final product.

Figures

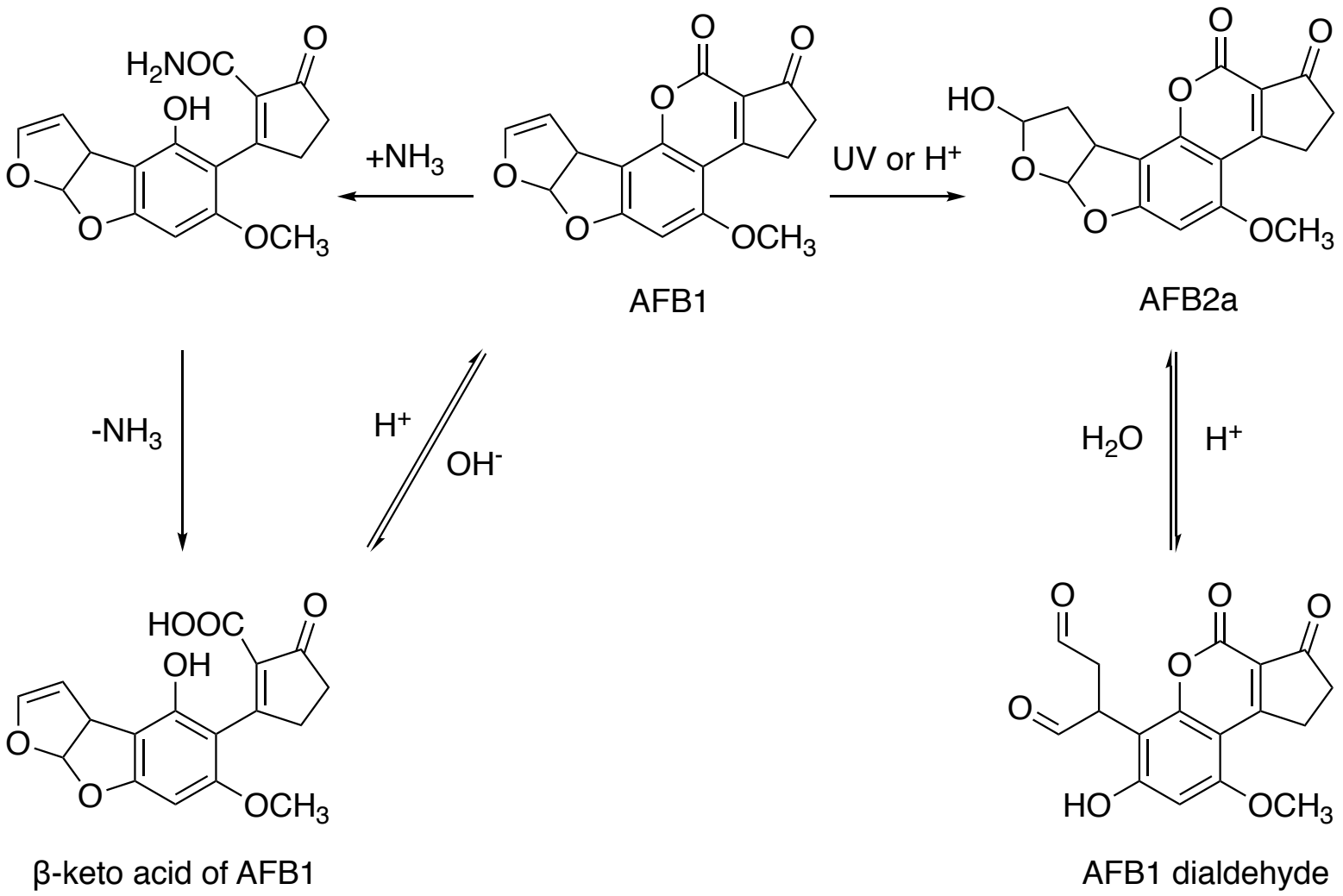

Figure 2.1 Proposed degradation pathways of AFB1.

CHAPTER 3

DETERMINING AFLATOXIN B1, B2, G1, AND G2 IN RAW PEANUTS BY REPLACING DERIVATIZATION WITH NORMAL-PHASE HPLC-FLD[1]

[1] Shen, M. H., & Singh, R. K. 2022. Submitted to *Food Control*, 3/5/2022.

Abstract

This study aimed at simplifying the conventional HPLC-FLD aflatoxin analysis procedure by combining immunoaffinity column (IAC) and normal-phase chromatography. The miscible issue of the eluate in the normal-phase solvent was solved by replacing methanol with acetonitrile as the eluent. The proposed mobile phase (toluene/ethyl acetate/methanol/formic acid, 90 mL/6 mL/2 mL/2 mL) considerably enhanced aflatoxin fluorescence by reducing quenching effect from the solvent. Each analysis needed less than 25 mL of solvent to complete. Time-consuming steps in the sample preparation procedure, i.e., nitrogen evaporation and derivatization, were not needed. The performance of using the proposed method in analyzing raw peanuts is better than the official method (HPLC-FLD combined with post-column iodine-derivatization) and meets the US FDA's standard. The method can determine AF concentration in raw peanuts contaminated with more than 4 ng/g total aflatoxins (containing 2 ng/g aflatoxin B1).

Introduction

Aflatoxins (AF) have been identified as carcinogens for poultry and humans, and thus different methods for their detection or quantification have been developed (Wacoo et al., 2014). Among those methods, thin layer chromatography (TLC) was widely used for AF detection due to its low cost and simplicity of analysis, but for accurately quantifying AF in low concentrations with acceptable repeatability, costly equipment, as well as experienced operators were needed (Zhang & Banerjee, 2020). Another method, enzyme-linked immunosorbent assay (ELISA) is easy to handle, but a false-positive or false-negative result was usually obtained (Rodríguez Velasco et al., 2003). Using immunoaffinity column (IAC) as a sample clean-up process followed by fluorometry is an appropriate option for sieving contaminated crops in the food industry, yet the AF concentration is frequently overestimated due to the interference from the sample matrix (Rahmani et al., 2010; Trucksess et al., 1991). Additionally, fluorometry cannot determine the concertation of each type of AF and hence is not suitable for measuring different types of AF (AOAC, 2002).

High-performance liquid chromatography (HPLC) combined with a fluorescence detector (FLD) could be an ideal option for those who would like to obtain sufficient information (i.e., the concentration of each type of AF) with good repeatability. LC combined with mass spectrometry (MS) is recognized as the most sensitive method for AF analysis, but the cost of the equipment and the subsequent maintenance is not affordable for most laboratories (Wacoo et al., 2014). Instead, FLD is an affordable option even though it is not as sensitive as MS. AF has a low UV absorption but a sufficient fluorescence emission at ppb levels, and therefore an FLD rather than UV detector is always used for AF analysis (Kok, 1994). However, the fluorescence emission of AF is considerably affected by the composition of the mobile phase and types of AF. For instance,

the fluorescence of aflatoxin B1 (AFB1) and G1 (AFG1) strongly diminishes with the presence of aqueous solvents (Kok, 1994). The phenomenon is also known as “fluorescence quenching” (Lakowicz, 1999).

To mitigate the quenching issue in an HPLC-FLD analysis, two types of methods are usually employed: one is using derivatization to enhance fluorescence response, the other is using selected solvents under the normal-phase condition (Kok, 1994). The former involves a fluorescence enhancing procedure such as pre-column or post-column derivatization. Pre-column derivatization uses trifluoroacetic acid (TFA) to hydrate the double bonds on the C8-C9 ring of AF before the sample injection step to obtain aflatoxin B2a (AFB2a) and G2a (AFG2a), which has a higher fluorescence emission under reverse-phase (Park et al., 1990). The major disadvantage of the TFA method is that AFB2a and AFG2a are unstable under reverse-phase, especially with the presence of methanol (Holcomb et al., 1992). Also, the evaporation step is time-consuming and may increase the random error of the analysis. Similarly, post-column methods add halogens, usually iodine or bromine, to the double bonds on the C8-C9 ring after AF passes the column for separation (Trucksess et al., 1991). The disadvantages of the post-column method are that the derivative reagents should be prepared daily, and an additional motor is required to pump chemicals for derivatization. Post-column photochemical derivatization, which hydrates AFB1 and AFG1 to AFB2a and AFG2a by exposing the eluate to UV after passing the separation column, was developed for mitigating the inconvenience of chemicals derivatization (Joshua, 1993). The method needs only an add-on in-inline UV reactor before the detector, but the parameters need to be optimized for maximizing AF hydration while preventing them from being decomposed by UV (Holcomb et al., 1992; Joshua, 1993). The transmittance of the UV reactor and the intensity decrease through time would affect the result (Papadopoulou-Bouraoui et al., 2002). Generally,

derivatization increases the random error, time, and cost of the analysis. A normal-phase system, toluene/ethyl acetate/methanol/formic acid (90 mL/6 mL/2 mL/2 mL), was proposed and modified for AF concentration determination (Leitao et al., 1988; Manabe et al., 1978). In the proposed method, fluorescence emission was significantly enhanced by introducing formic acid and replacing chloroform or dichloromethane with toluene, and the improvement does not require additional equipment (Manabe et al., 1978). The disadvantage of using the proposed normal-phase is the mobile phase should be stored in ice to stabilize the mixed solvent (Leitao et al., 1988).

Sample clean-up by IAC is an efficient (less than 10 min for each sample from raw peanut extraction) and affordable way (i.e., less than $8 for each Aflatest® column from Vicam, MA, U.S.) and hence has been prevalently employed in the industry and laboratories for AF analysis (Zhang & Banerjee, 2020). However, the recommended solvent used to elute the AF binding on antibodies is methanol (AOAC, 2000b, 2002), which is less miscible in the proposed normal-phase system than in an aqueous-based system, resulting in poor chromatography in the proposed normal-phase system (Fig. 3.1). Therefore, using an appropriate solvent to elute AF from the IAC is desired.

Accordingly, the combination of using IAC with an appropriate elution solvent and normal-phase chromatography can function as the post-column iodine-derivatization step to obtain sufficient sensitivity and repeatability in the current HPLC-FLD AF analysis procedure. In this study, we aimed at developing an AF quantification method using IAC as the sample clean-up process followed by normal-phase HPLC-FLD. The miscible issue of the eluate, the shelf-life of the mobile phase, and the parameters for optimizing the normal-phase chromatography were investigated. The validation of the method was also performed on raw peanut samples.

Material and Methods

This study was performed by a trained operator in the analytical laboratory certified with the recognized International Standard: ISO/IEC 17025 (Agricultural and Environmental Services Laboratories, University of Georgia, 2300-2400 College Station Road, Athens, GA 30602, GA 30602, USA.

Reagents

Methanol (MeOH), formic acid, toluene (Tol), and ethyl acetate (EtOAc), were all HPLC grade and purchased from Fisher Scientific (Rockingham, NH, U.S.). Crystal AFB1, crystal aflatoxin B2 (AFB2), crystal AFG1, crystal aflatoxins G2 (AFG2), certified reference material (CRM) AFB1 (Lot LRAC8734), CRM AFB2 (Lot LRAC8736), CRM AFG1 (Lot LRAC8735), CRM AFG2 (Lot LRAC4858), $MgSO_4$ anhydrous, NaCl, and acetonitrile (MeCN) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). AflaTest® (type P) IAC were purchased from Vicam (Milford, MA, U.S.).

AF stock solution and standard solution

Dry AF was prepared into stock solutions in MeOH with a concentration at 10µg/mL by AOAC method 971.22 (AOAC, 2000a). The method was also used by Sigma-Aldrich for CRM AF standard preparation (Supelco, 2017). The stock solution was calibrated by AF standard solution. The standard solution was prepared by reconstituting the CRM AF solution into MeCN/Tol (60 mL/40 mL) and diluted to desired concentrations. Four AF standard solutions were mixed at the ratio of AFB1/AFB2/AFG1/AFG2, 4 ng mL^{-1}/1 ng mL^{-1}/2 ng mL^{-1}/1 ng mL^{-1}, for making a calibration curve.

Extraction solution

MeOH-deionized water (DI water) (70 mL/30 mL) was used to extract AF from peanuts.

Mobile phase

The mobile phase was prepared by the modified composition (Leitao et al., 1988). Tol/EtOAc/MeOH (45 mL/3 mL/2 mL) were well mixed as Solution A; Tol/EtOAc/formic acid (45 mL/3 mL/2 mL) were well mixed as Solution B. Solution A and B were mixed in the HPLC pump at a ratio of 1 mL:1 mL. Both solutions can be stored in the dark at room temperature for two weeks. The mixing should only be done in the HPLC pump for preventing a spontaneous reaction.

Sample preparation

Non-blanched, shelled, runner-type peanuts (*Arachis hypogaea L.*) contaminated with less than 2 ng/g of AF were obtained from Golden Peanut Corporation (Alpharetta, GA, U.S.), vacuum sealed, and stored at 4˚C until used. For method validation, 150 g peanuts were loaded into a 500 mL Erlenmeyer's flask and spiked with 3.75 mL AF stock solutions to contain 2.0-0.5-1.0-0.5 ng/g, 4.0-1.0-2.0-1.0 ng/g, 10.0-2.5-5.0-2.5 ng/g, and 20.0-5.0-10.0-5.0 ng/g of AFB1-AFB2-AFG1-AFG2 followed by drying as described in our previous work (Shen & Singh, 2021). AF stock solutions were prepared by mixing 4 types of AF and diluting with MeOH to contain 80-20-40-20 ng/mL, 160-40-80-40 ng/mL, 400-100-200-100 ng/mL, and 800-200-400-200 ng/mL of AFB1-AFB2-AFG1-AFG2.

Sample extraction and IAC clean-up

AF was extracted from peanuts by the modified method AOAC 991.31 (AOAC, 2002). About 25 g of peanuts (specific weight was recorded to the second decimal) were blended (Blender model WF2211214, Waring corporation, Torrington, CT, U.S.) with 125 mL extraction solution and 5 g NaCl at the high speed for 2 minutes. The blended paste was passed through a fluted filter paper (24 cm, Fisher Scientific, Rockingham, NH, U.S.). The obtained first-filtration (12 mL) was

mixed with 20 mL DI water and filtered by a 1.5 μm glass microfiber filter (Whatman® 934-AH, GE Healthcare, Chicago, IL, U.S.). The second-filtered filtration (20 mL) was passed through an AflaTest® IAC by an air pump (Vicam, MA, U.S.) at a rate of one drop per second. The column was then washed by passing 20 mL of DI water at the same rate. After DI water was passed, the air was continuously pumped into IAC until no liquid was coming out. AF was then eluted by passing 0.45 mL MeCN into a test tube. Eluting of MeCN was completed by gravity without the aid of the air pump. If the IAC was blocked by bubbles, a syringe was used to force MeCN to pass the column until the bubbles were excluded, and then the rest of MeCN was passed by gravity as previously described. Forcing all MeCN to quickly pass through the IAC will cause a low AF recovery rate. When all MeCN was passed, a syringe was used to manually pump 10 mL of air into the IAC to exclude the residual MeCN. The test tube was then submerged in an ice bath. $MgSO_4$ anhydrous (0.2 g) and Tol (0.3 mL) were added to the test tube and vortexed until the eluate became clear. The eluate was pipetted to a 2.0 mL sample vial with a 0.4 mL insert (Fisher Scientific, Rockingham, NH, U.S.). $MgSO_4$ formed larger particles and precipitated after absorbing the water from the eluate, so an additional filtration was not necessary if the operator could carefully avoid the particles while transferring the eluate.

The final concentration of AF in peanuts can be obtained by the formula:

$$W = 25\ g \times (12\ mL/125\ mL) \times (20\ mL/32mL) = 1.5\ g \quad (1)$$

$$\text{Aflatoxin (ng/g)} = A \times E \times (1/W) \times (S/25) = \text{aflatoxin per 2 g of peanut} \quad (2)$$

where W = peanut weight equivalent to the filtrate passed through the IAC (g), S = actual sample weight of peanuts (g, to the second decimal), E = the total volume of eluent, which includes eluting aflatoxin from IAC by MeCN (0.45 mL) and improving chromatography performance by Tol (0.30

mL), and A = concentration of AF in the eluate injected into the HPLC (ng/mL). Total AF was obtained by adding concentrations of four types of aflatoxin.

Fluorescence excitation and emission wavelength determination

The excitation and emission wavelength to obtain the highest fluorescence intensity from AF in the proposed mobile phase were firstly determined. Solution A (0.5 mL) and B (0.5 mL) from Section "Mobile phase" and AF stock solution (1 µL) were mixed to simulate the condition in FLD. The mixture was transferred to a 1.5 mL quartz cuvette and loaded into a Cary Eclipse Fluorescence Spectrophotometer® (Agilent, Santa Clara, CA, U.S.). The sample was excited with wavelengths from 300 to 400 nm with a 5 nm increase, whereas the emission wavelength was collected from 400 to 500 nm with a 5 nm increase.

HPLC-FLD

Chromatography analysis was performed on Agilent 1260 Infinity HPLC system (Santa Clara, CA, U.S.) with a silica column (LC-Si, 5 µm, 25 cm × 4.6 mm, Supelco, Bellefonte, PA, U.S.) by the modified methods (Leitao et al., 1988; Manabe et al., 1978). The column was heated to 30˚C for accelerating separation. When not in use, the column was saturated with Tol/EtOAc (90 mL/10 mL). Before analysis, the column was flushed with Solution A and B described in Section "Mobile phase" (1 mL:1 mL) at 0.5 mL/min for 30 min followed by 1.5 mL/min for the other 30 min. The analysis was performed with a constant flow rate at 1.5 mL/min with the flushing of Solution A and B (1 mL:1 mL). After analysis, the column was washed with Tol/EtOAc (90 mL/10 mL) at 0.5 mL/min for 30 min followed by 1.0 mL/min for the other 30 min. The sample injection volume was 40 µL. The excitation and emission wavelength of FLD were set at 365 and 425 nm, respectively. The sampling rate was 2.31 Hz, and the PMT gain was 10. The room temperature was kept at 20 ± 2˚C.

Method validation

The validation process included examination of linearity, the limit of detection (LOD), and the limit of quantification (LOQ) of the calibration curve, according to the standard of European Pharmacopoeia (Shrivastava & Gupta, 2011). Accuracy and precision were determined by performing the analysis on AF spiked peanuts. Each AF concentration level was performed 3 replicates on three different days with an additional 2 replicates on one selected day (Table 3.1). The result was validated with the AOAC method 991.31: solution fluorometry with bromine derivatization (SFB) as described in our previous work (AOAC, 2002; Shen & Singh, 2021).

Peanut inoculation

Fresh peanuts with less than 2 ng/g of AF contamination were inoculated with *Aspergillus nomius* (NRRL 6108) and used to test the proposed method. The spore suspension (18 mL) solution containing 10^6 spores/mL was added to 150 g of peanuts and incubated at 30˚C for two days.

Results and Discussion

Optimization of the analysis process

In previous studies, four solvents, Tol-EtOAc-MeOH-formic acid, were mixed before being pumped into the HPLC system (Leitao et al., 1988; Manabe et al., 1978). Pre-mixing solvent solves the problem that the miscibility of MeOH and formic acid in Tol is not as good as when mixing in aqueous solvents such as water. We tried to mix four types of solvents in the pump of HPLC, but poor chromatography results were observed due to a non-uniform mobile phase. Additionally, the pre-mixed solvent was not stable since the formic acid can act as a dehydration reagent and catalyze formic acid and MeOH to undergo spontaneous esterification. In our observation, a considerable amount of water was generated when the pre-mixed solvent was stored at room temperature for two days. Therefore, in the previous studies, the pre-mixed solvent should

be prepared daily and kept in an ice bath while performing analysis. Alternatively, we found that these pre-mixing solvents but separating MeOH and formic (see Section “Mobile phase”) can considerably prolong the shelf life of the mobile phase, largely reducing the use and the waste of the solvent. No significant change in chromatographic property was observed when Solution A and B stored in the dark under room temperature for two weeks were used.

The miscibility issue occurred when injecting the eluate into the normal-phase system. The AF in MeOH showed poor chromatography due to the low miscibility of the eluate in the mobile phase (Fig. 3.1). Evaporating the eluate using nitrogen followed by reconstituting it in the higher miscible solvent is a solution, but it takes more time and labor. We attempted to replace MeOH with MeCN as an eluent to facilitate the process. In the proposed procedure (see Section “sample extraction and IAC clean-up”), only a few more time and labor were involved for passing MeCN through the IAC by gravity and for adding $MgSO_4$ anhydrous to remove water from the eluate.

Additional Tol was added to the eluate before analysis to improve the chromatography. Without the presence of Tol, a serious pre-peak shouldering was observed, as shown in Fig. 3.2A. The result demonstrated that adjusting the portion of Tol to 40% (mL/mL) into the eluate before chromatography can significantly improve the peak shape and thus facilitate the subsequent peak area integration process.

The effect of eluate injection volume on change of peak shape was also evaluated. In the previous studies, injection volume could be as high as 50 µL or even 100 µL (Rahmani et al., 2010; Trucksess et al., 1991). However, the high injection volume may cause a wider peak or even peak overlap. We tested different injection volumes from 10 to 50 µL, and the corresponded chromatograms are shown in Fig. 3.2B. As can be seen, a similar peak shape indicated the column can endure a higher injection volume. Nonetheless, the peak width is considerably increased and

may overlap with each other if the sample has a higher AF concentration. A 40 µL of injection volume was used in this study due to the peak-overlapping issue and obvious pre-peak shoulders observed in a 50 µL of injection.

The optimum excitation and emission wavelengths of FLD were also determined. Despite the condition was described in the previous study (Manabe et al., 1978), the authors did not specifically state whether the selected wavelengths were optimized. Fig. 3.3 shows that setting excitation and emission wavelengths at 365 and 425 nm for AFB1, respectively, has the highest fluorescence intensity, which is in accordance with Manabe et al. (1978). For other types of AF, the optimum wavelengths are different from AFB1. Since AFB1 is the predominant toxin and the most toxic among the four, the current wavelength setting is preferred (Van Egmond & Jonker, 2004). The optimum wavelengths might be modified if other types of AF are of more interest.

Validation of the process

Linearity and sensitivity

Fig. 3.4 shows the chromatograms of AF standards for calibration using the optimized parameters described in Section "Optimization of the analysis process." No significant abnormal peak property can be observed except the baseline drift occurred at a higher AF concentration, but the issue did not significantly influence the result. The linearity and the sensitivity of four types of AF are acceptable ($R^2 > 0.999$) at the total AF concentration from 2.50 to 320.00 ng/mL by the proposed methods (Table 3.2). The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC are determined at the S/N ratio greater than 3 and 10, respectively, according to European Pharmacopoeia (Shrivastava & Gupta, 2011). The method was chosen because of the simplicity to be employed in the chromatographic analysis.

Accuracy and precision

The accuracy (recovery rate) and precision (relative standard deviation, RSD) of our results (Table 3.1) validated the use of the proposed method. The method has the potential to meet US FDA's criteria which requires a reproducible accuracy between 80% to 120% and a precision less than 20% (Shrivastava & Gupta, 2011). Most of our results meet that standard except the analysis performed on Day 3, total AF at 4 ng/g level and AFG2 at all levels. The higher recovery rate and RSD of the Day 3 sample of the lowest level indicate that the contamination already existed in the peanuts before we used them. We found that the fresh peanuts were occasionally contaminated with about 5 to 10 ng/g of AF. Even though the concentration does not exceed the US FDA's regulation (US FDA, 2018), the contamination is sufficient to affect our final results. The low recovery rate of AFG2 is due to the inherently low affinity of the antibody to the analyte (AOAC, 2000c; Trucksess et al., 1991). The manufacturer, Vicam, confirmed the issue of low affinity to AFG2 and suggested using an Aflatest® WB column to mitigate the issue. The performance of the proposed method is even better than that of the official method, which employed the post-column iodine-derivatization method (AOAC, 2002; Trucksess et al., 1991). The other parameters for performance evaluation such as extraction rate can be referred to the AOAC method 991.31 since the sample preparation procedure in our method is similar to that in the official method (AOAC, 2002).

Our proposed method shows not only a higher selectivity of different types of AF (Fig. 3.4) but also a better LOQ. The performance of RSD_R reveals that the LOQ of analyzing whole peanuts by our method can be as low as 4 ng/g of total AF, or 2 ng/g of AFB1. The LOQ is much less than that of AOAC methods 991.31, which is 10 ng/g (AOAC, 2002). The LOQ meets the action level of AF in raw peanuts (<20 ng/g of total AF) regulated by the US FDA and can be further lowered

down to fulfill the requirements for ready-to-eat peanuts (<4 ng/g of total AF and <2 ng/g of AFB1) regulated by European Union (Knutsen et al., 2018). According to the LOQ of the standard curve (Table 3.2), the theoretical LOQ in peanuts can be as low as 1.25-0.31-0.63-0.31 ng of AFB1-AFB2-AFG1-AFG2 per gram of peanut, or 2.5 ng of total AF per gram of peanut.

Validation of the proposed method

The results obtained by our proposed method were not statistically significantly different from those obtained by the SFB method, justifying the use of our method. Due to the non-selectivity of the SFB method, we used peanuts spiked with only AFB1 as the sample. The observed values of peanuts spiked with 4 ng/g, 10 ng/g, and 20 ng/g of AFB1 are 7.71 ± 0.29 ng/g, 11.96 ± 0.58 ng/g, and 17.18 ± 0.67 ng/g, respectively. The recovery rate observed in the SFB method at the 4 ng/g level largely exceed 100% and therefore was not included in the comparison. The influence comes from the peanut matrix, revealing that the SFB method was not recommended for AF concentration determination if the contamination level is lower than 10 ng/g (Trucksess et al., 1991). In our previous works, we found that the background value in peanuts was about 2 to 3 ng/g. Although the SFB method is more repeatable (RSD < 5%), the LOQ and the selectivity of AF limit its application.

Analysis of fresh peanuts and fungi inoculated peanuts

Samples from two batches of fresh peanuts and one fungus-inoculated peanut were analyzed. One of the fresh samples contained 4.89 ng/g of AFB1 and 0.46 ng/g of AFB2, while the other fresh sample contained 7.04 ng/g of AFB1 and 0.49 ng/g of AFG1. The inoculated sample contained 1.28 ng/g of AFB1 and 1.70 ng/g of AFG1. Even though AF was found in the purchased peanuts, the contamination level was lower than the US FDA's regulation (US FDA, 2018). Different types of AF were found in the purchased peanuts. AFB1 was found in all samples,

whereas AFB2 or AFG1 was occasionally observed in some samples, which is consistent with the study of Van Egmond & Jonker (2004). On the other hand, the fungus inoculated peanut shows a higher AFG1. The fungus, NRRL 6108, was isolated from wheat and usually produces the same amount of AFB1 and AFG1. The low concentration of AF in the inoculated peanuts could result from the short incubation time.

Conclusions

The results demonstrate that our method can completely replace the time-consuming derivatization step that is widely used in the current HPLC-FLD AF analysis. Our works successfully fit the eluate from the IAC for the normal-phase chromatography, prolong the shelf-life of the mobile phase, and enhance the fluorescence of AF to a sufficient level for analysis. Even though it is the trend to use reverse-phase in an HPLC analysis, a considerable amount of MeCN and halogens for AF analysis in the official methods are still needed. On the other hand, our proposed method required less than 25 mL of less-poisonous organic solvent for each analysis. The validation results showed that this method can detect the AF level lower than that set by the US FDA for raw peanuts. The LOQ just reaches the standard set by the European FDA, which is 4 ng/g for total aflatoxins, but it is possible to further lower the LOQ to fulfill the requirements by modifying parameters such as increasing injection volume of the eluate.

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Conflict of interest: none

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Tables

Table 3.1 Accuracy and precision analysis of spiked peanuts obtained by the proposed method.

Toxin type	Nominal concentration (ng/g)	Experimental concentration (ng/g)										Avg_r	SD_r	Avg_R	SD_R	$Recovery_r$ (%)	RSD_r (%)	$Recovery_R$ (%)	RSD_R (%)
		Day 1				Day 2				Day 3									
B1	20.0		17.84			17.32				20.39				18.51	1.65			93	9
B2	5.0		4.88			4.21				4.78				4.62	0.36			92	8
G1	10.0		9.04			9.75				11.65				10.14	1.35			101	13
G2	5.0		2.21			2.18				2.19				2.19	0.02			44	1
B1	10.0	11.15	9.51	11.09		7.49				10.17		10.58	0.93	9.28	1.92	106	9	93	18
B2	2.5	2.53	2.48	2.93		1.77				2.41		2.64	0.24	2.27	0.45	106	9	91	20
G1	5.0	5.37	5.31	5.81		4.06				5.44		5.50	0.27	5.00	0.81	110	5	100	16
G2	2.5	1.08	1.03	1.15		1.22				1.38		1.08	0.06	1.23	0.15	43	6	49	12
B1	4.0		4.49		4.90	4.46	3.41			4.52		4.26	0.77	4.42	0.14	106	18	111	3
B2	1.0		0.88		1.09	1.10	0.94			0.85		1.04	0.09	0.93	0.10	104	9	93	11
G1	2.0		2.00		2.45	2.02	1.77			2.13		2.08	0.34	2.07	0.06	104	16	104	3
G2	1.0		0.53		0.69	0.72	0.60			0.56		0.67	0.06	0.59	0.07	67	9	59	13
B1	2.0		2.08			1.97		1.74		2.02	3.65	2.47	1.03	2.17	0.26	124	42	109	12
B2	0.5		0.53			0.59		0.45		0.81	0.58	0.61	0.18	0.58	0.04	122	30	115	8
G1	1.0		1.18			1.08		0.97		1.11	1.25	1.11	0.14	1.13	0.05	111	13	113	5
G2	0.5		0.32			0.42		0.33		0.43	0.48	0.41	0.08	0.38	0.05	82	18	77	14

[R] represents samples obtained between days.

[r] represents samples obtained within a day.

Table 3.2 Calibration curve (n = 3) of aflatoxins standard generated by the proposed method.

Toxin Type	Range (ng/mL)	Slope	Intercept	R^2	LOD (ng/mL)	LOQ (ng/mL)	S/N ratio at LOD
B1	1.25 – 160.00	0.56 ± 0.03	-0.02 ± 0.16	>0.999	1.25	1	5
B2	0.31 – 40.00	1.04 ± 0.09	0.16 ± 0.45	>0.999	0.31	0.63	3
G1	0.63 – 80.00	1.43 ± 0.06	-0.17 ± 0.17	>0.999	0.63	1.25	4
G2	0.31 – 40.00	4.28 ± 0.21	-1.00 ± 0.18	>0.999	0.31	0.63	4

Figures

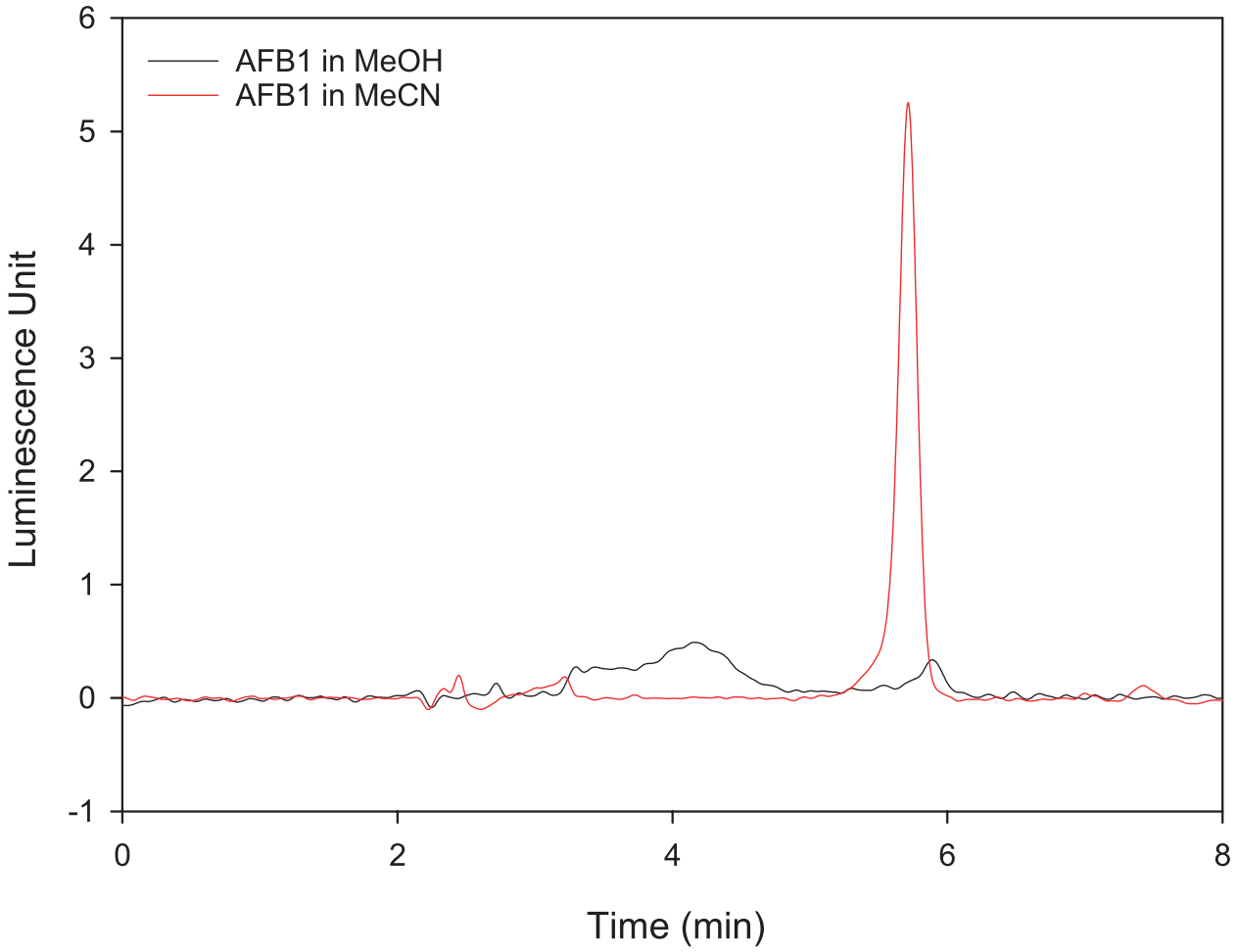

Figure 3.1 Chromatograms of aflatoxin B1 (AFB1) standard dissolved in MeOH and MeCN (100 ng/mL) under the proposed HPLC-FLD method.

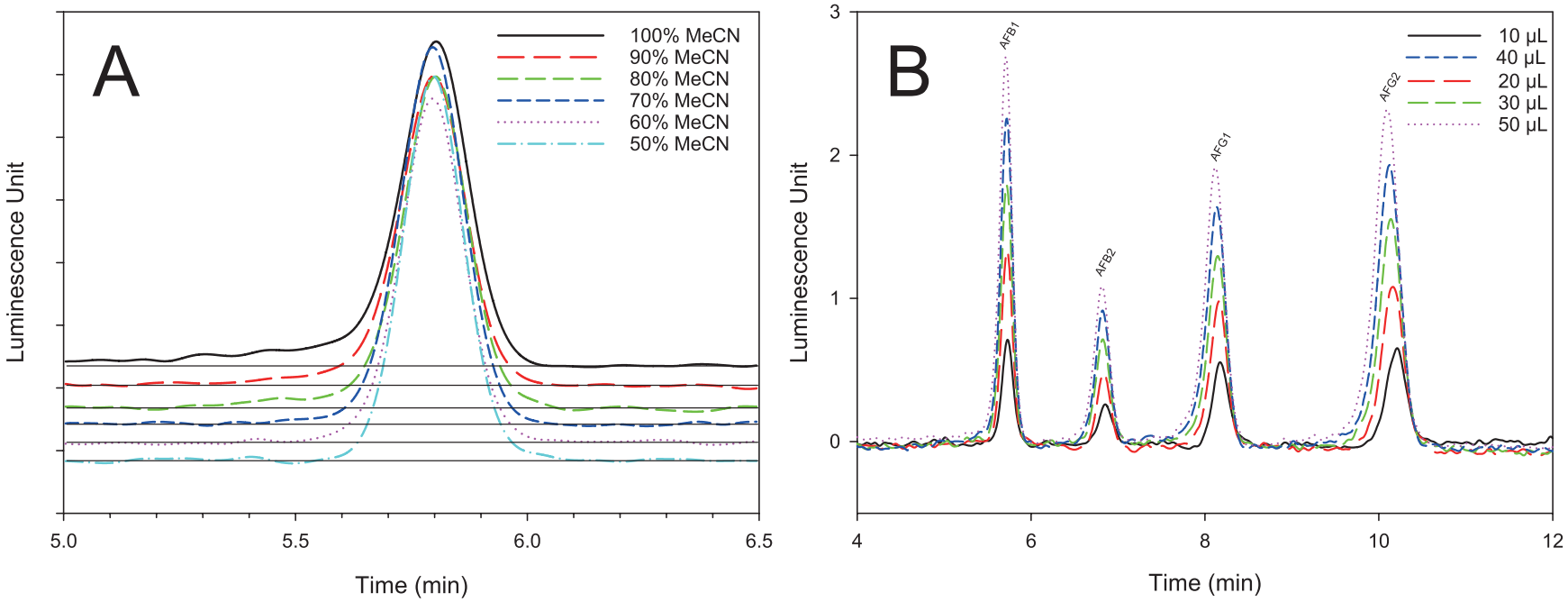

Figure 3.2 (A) Aflatoxin B1 (100 ng/mL) dissolved in solvents with different ratio of Tol/MeCN (mL/mL). (B) Different injection volumes of aflatoxins standard (B1:40 ng/mL; B2:10 ng/mL; G1: 20 ng/mL; G2 10 ng/mL) dissolved in Tol/MeCN (40 mL/60 mL).

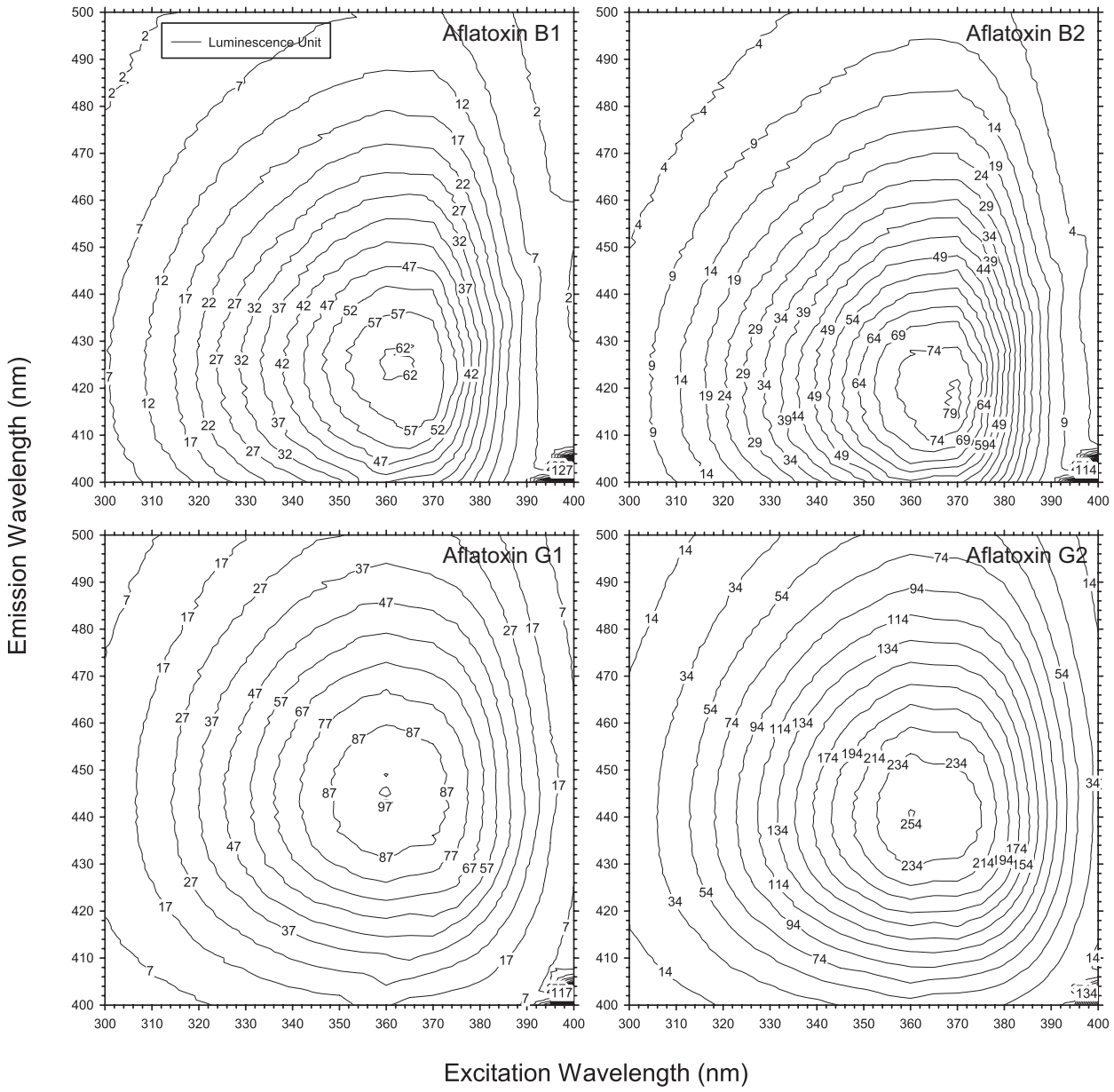

Figure 3.3 Fluorescence intensities of four types of aflatoxin in Tol/EtOAc/formic acid/MeOH (90 mL/6 mL/2 mL/2 mL) at different excitation and emission wavelengths.

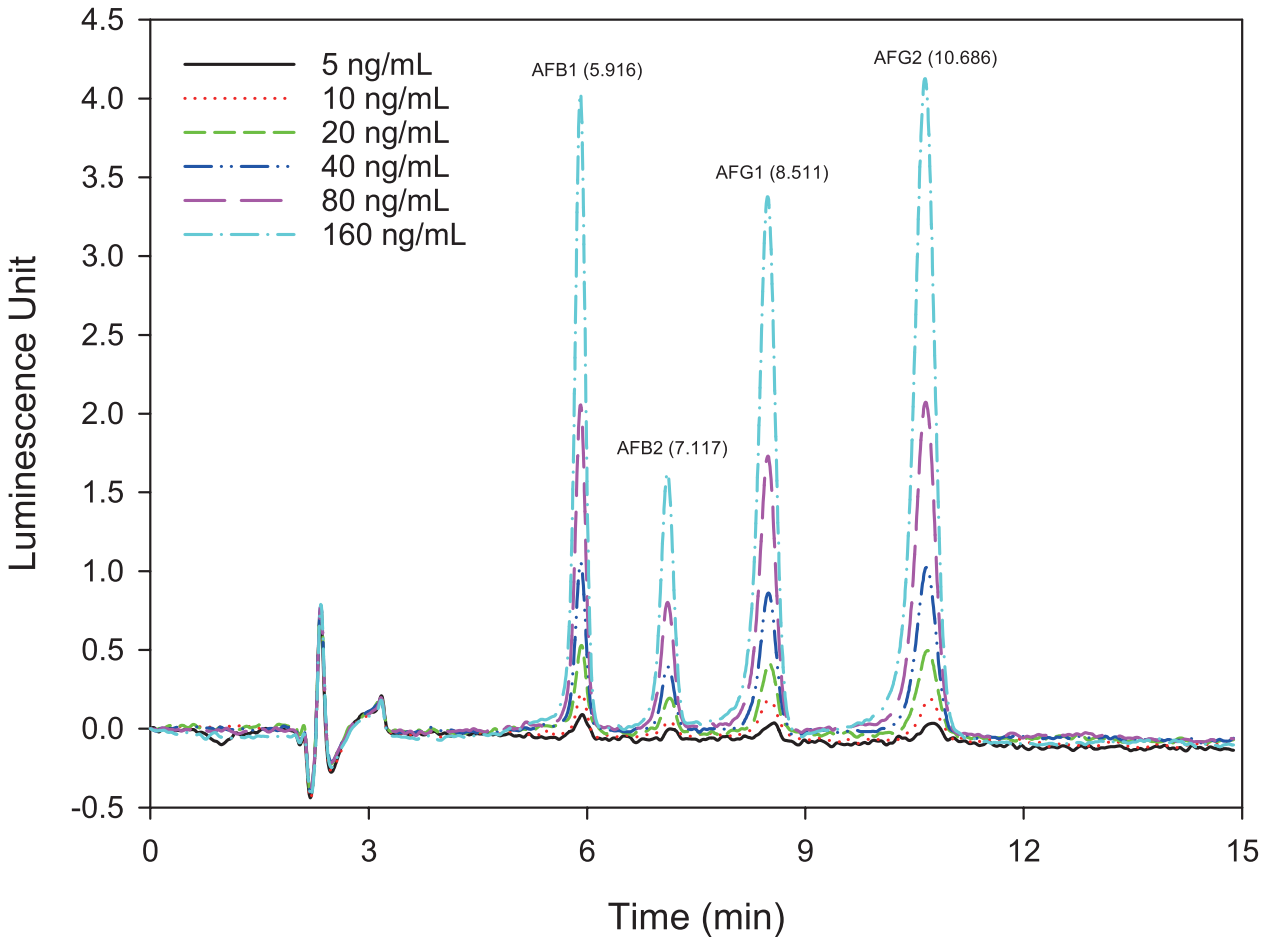

Figure 3.4 Chromatograms of different levels of aflatoxin mixture with a ratio, AFB1/AFB2/AFG1/AFG2, 4 ng/1 ng /2 ng/1 ng, for calibration under the proposed conditions (Section "Sample extraction and IAC clean-up" and "HPLC-FLD"). Numbers in the legend represent the concertation of total aflatoxins. (AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2)

CHAPTER 4

EFFECT OF ROTATING PEANUTS ON AFLATOXIN DETOXIFICATION BY ULTRAVIOLET C LIGHT AND IRRADIATION UNIFORMITY EVALUATED BY AGCL-BASED DOSIMETER[1]

[1] Shen, M. H., & Singh, R. K. 2020. *Food Control*. 120, 107533. Reprinted here with permission of the publisher.

Abstract

Ultraviolet irradiation (UV) has been used as a non-thermal processing method for food disinfection. In disinfecting solid foods such as peanuts, UV irradiation could be shaded or attenuated with increased distance, resulting in non-uniform dosage distribution. An affordable, rapid, and feasible method for measuring and improving irradiance distribution, however, is unavailable for detoxifying peanuts. In this study, a method was developed for rapidly quantifying UV dosage distribution on peanuts in a UV disinfection process. The darkening of the UV indicator, AgCl, was linearly proportional to the UV dosage from 0 to 120 mJ/cm2 delivered on peanuts. The uniformity of UV dosage distribution was described by measuring the color change of UV indicator at four points on two orthogonal axes of each peanut kernel. The UV indicator-coated peanuts were then rotated in a customized cylindrical chamber at different speeds. A rotation speed with a more uniform UV dosage distribution was determined and applied to an aflatoxin detoxification process. The results demonstrate that the UV uniformity was significantly improved when peanuts were rotated at 11 rpm in the cylindrical chamber. Furthermore, after irradiating with 2.3 mW/cm^2 UV-C for 2 hours, the aflatoxin B1 degradation rate increased from 60.8 ± 15.3 $pmol \cdot g^{-1}h^{-1}$ to 75.0 ± 10.9 $pmol \cdot g^{-1}h^{-1}$ in the peanuts rotated at 11 rpm, compared to those that were not rotated.

Introduction

Ultraviolet irradiation (UV) is a non-thermal technology for food disinfection and has been introduced in food manufacturing for years (Shama, 1999). Being an electromagnetic wave, UV carries radiant energy through space, inducing photochemical reactions of organic molecules such as the degradation of aflatoxins (Patras et al., 2017). This feature allows UV to disinfect either harmful microorganisms or hazardous chemicals in foods. Free radicals such as reactive oxygen species and their derivatives produced from oxygen or hydrogen peroxide by UV can react with chemicals, such as mycotoxins in foods (Karlovsky et al., 2016). UVC, also known as germicidal UV, is mostly used in a disinfection process due to its higher disinfection efficiency (Shama, 1999). One of the mycotoxins, aflatoxin (AFT), has been recognized as a significant global food contaminant that is carcinogenic and mutagenic to humans and some animals (Craig, 1973; Horn, 2003). UV has been used to detoxify AFT in foods, especially nuts (Altuğ et al., 1990; Basaran, 2009; Diao et al., 2015; Garg et al., 2013; Jubeen et al., 2012). In these studies, most of the efforts were focused on UV intensity, exposure time, and degradation kinetics of AFT.

UV can disinfect solid and liquid foods, however, with different efficiency levels. The UV irradiation drastically attenuates as the opacity of the material increases. Namely, UV has a much shorter penetration depth in solid foods than in liquid foods (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Lim & Harrison, 2016). In most liquid foods with low turbidity or fewer suspended solid particles, UV has a higher transmission rate and disinfection efficiency (Koutchma, 2008; Magzoub et al., 2019; Yousef & Marth, 1985). However, most solid foods and liquids with high turbidity or suspended particles will considerably obstruct the transmission of UV (Koutchma, 2008).

Previous studies on disinfecting solid foods using UV have shown that not all areas of the solid can be exposed uniformly to the incident irradiation, causing lower disinfection efficiency (Fan et al., 2017; Lim & Harrison, 2016). The AFT detoxification efficiency could be higher if the UV dosage delivered on the surface of objects was uniformly distributed (Altuğ et al., 1990; Jubeen et al., 2012). A similar conclusion was made in another study that eliminating the shading by rotating the solids can enhance the degree of pathogen reduction (Fan et al., 2017). Conceivably, creating uniform UV dosage distribution on the surface of solid foods should be able to increase the reduction of AFT.

Irradiating UV dosage uniformly over the entire surface of foods is another challenge while developing UV disinfection equipment. The light intensity follows the inverse-square law, and therefore, it will drastically attenuate as the distance between the subject and the UV light source increases. That is, the study calibrating UV intensity with an insufficient amount of sampling points could underestimate the AFT detoxification efficiency due to non-uniform disinfection (Garg et al., 2013). Multiple-location calibration by radiometers is a solution but is time-consuming. Additionally, it is difficult to install multiple sensors on a moving object transported by a conveyor belt in a continuous UV disinfection process (Lim & Harrison, 2016).

As described above, mitigating non-uniform UV dosage distribution during the process is a concern when applying this technology to disinfect solid foods. To assess UV dosage distribution, chemical actinometers and bio-dosimeters were introduced (Horneck et al., 1996; Kuhn et al., 2004). However, additional analyses were needed to determine UV dosage, such as measurement of concentration for actinometers or spore incubation for bio-dosimeters, limiting the application for large dosage measurements. Further, these indicators may be difficult to be fixed stably on solid foods with an irregular shape. Therefore, we chose AgCl photography emulsion as the UV

dosage indicator because of its versatility. The emulsion can be easily coated on the surfaces of the solid food, and the corresponding dosage can be obtained by measuring the color change of the emulsion after UV treatment (Osterman, 2007).

In this study, we first aimed to develop a feasible method for quantifying UV dosage uniformity using whole peanut kernels as a model. Then the non-uniformity of incident UV irradiation will be leveled by rotating peanuts, and finally the UV dosage distribution will be determined by the proposed uniformity quantification method. Eventually, the effect of leveling UV uniformity on detoxifying aflatoxin B1 (AFB1) in contaminated peanuts would be evaluated. The specific objectives are: to (1) investigate the non-uniformity of UV caused by different distance, (2) build a UV dosage profile based on the color change of AgCl photography emulsion coated on peanut surface, (3) determine the parameter of rotating peanuts for leveling UV dosage distribution by examining the pattern of the color change of AgCl emulsion, and (4) and evaluate the effect of leveling UV uniformity on AFB1 degradation in peanuts.

Materials and Methods

Sample and reagents

Non-blanched, shelled, runner-type peanuts (*Arachis hypogaea L.*) were obtained from Golden Peanut Corporation (Alpharetta, GA, USA) and were stored at 4°C until used. $AgNO_3$, NaCl, porcine gelatins (low strength gel: strength 90-110 g and high strength gel: strength 300 g), $CrK(SO_4)_2 \cdot 12H_2O$, ethanol, NaS_2SO_3, $NaCO_3$, and AFB1 were ACS Reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, U.S.). Methanol (HPLC grade) was purchased from Fisher Scientific (Rockingham, NH, U.S.). AflaTest® immunoaffinity column and developer stock solution (0.03% bromine) was purchased from Vicam (Milford, MA, U.S.).

The procedure of photography processing

The photography processing procedure was modified from Mark's procedure (Osterman, 2007). Since the emulsion is sensitive to light, the whole procedure described in this section was performed in a darkroom. The chemical reactions involved in this photography processing procedure are shown below:

The first step is the formation of AgCl particles, which are sensitive to UV.

$$AgNO_{3(aq)} + NaCl_{(aq)} \rightarrow NaNO_{3(aq)} + AgCl_{(solid)} \quad (1)$$

In the next step, the AgCl particles are mixed with an emulsifier (gelatin) and coated on the peanuts. UV triggers the formation of Ag particles, which become black in color.

$$AgCl_{(s)} + UV \rightarrow Ag_{(s)} + 1/2\ Cl_{2(g)} \quad (2)$$

Finally, the un-reacted AgCl particles are washed out. The UV dosage was "recorded" as a color change on the surface of the peanut.

$$AgCl_{(s)} + 2Na_2S_2O_{3(aq)} \rightarrow Ag(S_2O_3)_2{}^{3-}{}_{(aq)} + Cl^-{}_{(aq)} + 4Na^+{}_{(aq)} \quad (3)$$

The emulsion was prepared by the following steps: To prepare solution A, 2.04 g of NaCl and 8.00 g of gelatin mixture (1.00 g low strength gel and 7.00 g high strength gel) were completely dissolved in about 40.0 mL of 60°C water. To prepare solution B, 5.10 g of $AgNO_3$ was completely dissolved in about 40.0 mL of 60°C water. The emulsion was obtained by mixing the solution A, solution B, 1.5 mL of 5% (w/v) chromium potassium sulfate solution, and 2.0 mL of 95% ethanol. The volume of the mixture was then adjusted to 100.0 mL and kept at 60°C until used. The emulsion should only be prepared right before use because it starts hardening after the addition of chromium potassium sulfate solution.

Before coating with the emulsion, peanut kernels with partial skin or skin with too dark or too light color were sorted out by visual inspection to minimize the interference of background color. After sorting, peanuts were immediately coated with the emulsion. A batch of about 1000 g

sorted peanut kernels was loaded into a ball-shape container with 33 cm in diameter (Kaytee Run-about ball, Kaytee Products, Inc., Chilton, WI, U.S.) and rotated at 30 rpm. The photography emulsion was then transferred into a spray bottle (The Clorox Company, Oakland, CA, U.S.) and sprayed on the rotating peanuts in the ball-shaped container. About 30.0 g photography emulsion was sprayed on each batch of peanuts. The peanuts were rotated until the emulsion was covered on all kernel surfaces and hardened. The rotating process for the emulsion coating, cooling, and hardening took about 2 to 3 min to complete, depending on the room temperature and moisture content in the air. A longer rotating time is not recommended since over-rotating would damage the coating. Coated peanut kernels were then fully dried, protected from light, and stored at 4°C for up to 1 week.

UV-irradiated peanut kernels were soaked and delicately stirred in a 100 mL beaker containing 30.0 mL of fixer solution (15% (w/v) of $Na_2S_2O_3$ and 2% (w/v) of Na_2CO_3 mixture) for 2 min to wash out any residual AgCl. Fixer-washed peanut kernels were then mildly scooped up from the fixer, delicately rinsed with deionized water, and fully dried for later color measurement. The peanuts were not exposed to light before the fixer-washing step.

Measurement of UV intensity

UV intensity was measured by ILT800-UV CureRight® radiometer (Peabody, MA, U.S.). The desired UV intensity was set by adjusting the distance between the peanut and the UV light source. The UV lamps were warmed up for 10 min before use. The desired intensity of the UV irradiation was determined by taking the average intensity within a 5-minute measurement.

Measurement of peanut color

A colorimeter, Minolta CR-300 (Minolta Camera Co., Osaka, Japan), was used to measure L*, a*, and b* values (CIELAB color space) on each peanut. The diameter of the aperture used in

this study was 11 mm, which allowed focusing on the area with minimum interference from the area of no interest. Color measurement was performed by taking the color information at the sampling point on a peanut fixed on a stick holder with model clay (Figure 4.1). Each observed value was obtained by taking the average of three readings from the same sampling point.

UV dosage profile based on color change

A UV dosage profile was constructed according to the color change of the emulsion-coated peanuts after UV treatment. A batch of 10 emulsion coated peanuts was placed under the center of UV lamps and irradiated by 0.5 and 1.0 mW/cm^2 of UV. The UV irradiating times for 0.5 mW/cm^2 irradiation were 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 seconds, whereas the times for 1.0 mW/cm^2 irradiation were 0, 40, 80, and 120 seconds. Because the surface of the peanut is not flat, each area on a static peanut would not receive the same level of UV dosage. Therefore, only the color information of the area faced and closest to the UV lamps (usually the top of the peanut) was obtained for constructing the dosage profile. To simplify the evaluation process, only L* value was chosen by the principal component analysis (PCA) as the dosage indicator for the UV uniformity experiments. An unscaled matrix was used in PCA for sieving out the variable, which was the most sensitive to the change of UV dosage.

UV equipment

Peanuts were loaded into the customized UV equipment (Figure 4.2) built in the instrument shop at the University of Georgia. The equipment has a cylindrical chamber, which was 30 cm in diameter and 30 cm in length. The chamber was made of 316 stainless steel. The inside surface of the stainless-steel chamber was coated with P100 (ISO 6344 standard) stainless steel particles for increasing friction to facilitate the rotation of peanuts along its longitudinal axis. The rotation speed of the chamber can be precisely adjusted by the speed control motor (DSCI5060UV, ORIENTAL

MOTOR CO., LTD, Japan). A UV lamp holder located inside the chamber can hold two lamps. The UV intensity can be adjusted by changing the distance between the UV lamps and the sample loaded in the chamber. A low-pressure UVC lamp (model GPH357T5L/4Pins, 17 W, Light Sources Inc., Orange, CT, U.S.) with maximum emitting power at wavelength 254 nm was used in this study. The number of mounted lamps was determined according to the UV intensity required. A quartz sleeve protected the UV lamps from contamination with the food samples.

Determination of rotation parameters by evaluating UV uniformity

A batch of about 100 g emulsion-coated peanuts was loaded into the stainless-steel chamber of the UV equipment. The UV intensity was set at 0.5 mW/cm^2. The rotation speeds were 0, 2, 11, and 20 rpm, and the rotation times were 1, 2, 3, 4, 6, and 15 min. A rotation speed higher than 20 rpm was not recommended as the peanut kernels began to tumble over the other kernels when the rotation speed exceeded 20 rpm. Ten peanut kernels were randomly sampled from each treated batch for color measurement. The L* values were obtained by measuring four endpoints on two orthogonal axes on the surface of each sampled peanut (Figure 4.1). The influence of peanut weight loaded in the rotation chamber on UV uniformity was also examined. Three loading weights, about 100 g, 200 g, and 300 g of peanuts for a batch, were tested. Duplicate samples were used for each treatment.

Quantification of UV irradiation uniformity

To quantify the uniformity of UV irradiation, here we defined 2 indicators:

(i) *Average of L* values* of four axial points in a peanut: this indicator describes the average UV dosage received on the whole peanut surface. A higher *Average of L* value* indicated a higher UV dosage received by a peanut, and vice versa. The standard deviation of a group of *Average of L* values* can be used to describe the uniformity of UV to some extent but not completely. The

non-uniform UV irradiation within each peanut could be neglected if only this indicator is evaluated. Additionally, a statistical bias from the background color of each peanut cannot be eliminated and thus it will affect the results.

(ii) *Variance of L* values* of four axial points in a peanut: this indicator describes the difference of UV dosage received between the four points on a peanut. A greater *Variance of L* value* indicates a more non-uniform UV dosage distribution on peanut surface, and vice versa. This indicator can eliminate the statistical bias caused by the background color of each peanut. The non-uniformity in each peanut can be represented.

These two indicators are complementary and need to be evaluated at the same time. By evaluating these two indicators, the parameters for improving UV uniformity when rotating peanuts can be determined.

Preparation of artificially spiked AFB1 peanuts sample

The AFB1 stock solution with a concentration of 10.4 μg/mL used in this study was prepared by dissolving AFB1 crystal in methanol and quantifying by the procedure of AOAC method number 971.22 (AOAC, 2000). The stock solution should be stored at 4°C in the dark before use (Diaz et al., 2012).

For preparing artificially spiked peanuts, peanuts were firstly peeled by a peeling machine described in another study (Kettler et al., 2017). Our preliminary experiments indicated that peeled peanuts had a higher AFB1 reduction percentage under UV irradiation, compared to the unpeeled peanuts. A 17.5 mL of 10.0 μg/mL AFB1 diluted solution was added to about 500 g of peeled peanuts and mixed in a 2000 mL Erlenmeyer flask. The theoretical concentration of AFB1 in peanuts should be 350.0 ng/g. The Erlenmeyer flask was then tilted to about 45 degrees and rotated along its axis at about 30 rpm by hand until methanol evaporated. The level of remaining methanol

in peanuts was checked by weighing the flask. Airflow was sent into the flask during rotation for assisting methanol evaporation. Spiked peanuts were stored for up to a week before use. Our preliminary spiking test demonstrated that the above procedure could minimize the variation of AFB1 concentration in the same batch of peanuts. The actual AFB1 concentration in artificially spiked peanuts was 278.6 ± 21.7 ng/g (n = 3).

The parameters (rotation speed and peanut weight), which leveled the UV dosage distribution to the maximum extent, were applied to detoxify artificially AFB1 spiked peanuts. To minimize the damage of the peanut surface by rotation, a cycle composed of a long-term UV irradiation (30 min) at the first and a short-term rotation (1 min) at the end was set. A 4-cycle (total of 2 h) process was performed and compared with the control treatment. In the control treatment, peanuts were first treated with the same short-term rotation (total 4 min) without UV exposure, then irradiated with UV for 2 h. More intensive UV (2.3 mW/cm^2) was set for this experiment to accelerate the detoxifying process. The experiments were done in triplicate.

Aflatoxin assay

The AFB1 concentration was determined by the modified AOAC method 991.31 (AOAC, 1995). About 25 g of peanuts (specific weight was recorded to the second decimal) were blended (Blender model WF2211214, Waring corporation, Torrington, CT, U.S.) with 125.0 mL 70% methanol (v/v) and 5.00 g of NaCl at the high-speed level for 2 min. The blended paste was then passed through a fluted filter paper (24 cm, Fisher Scientific, Rockingham, NH, U.S.). A 15.0 mL of filtration was mixed with 30.0 mL of deionized water and filtered by a 1.5 µm glass microfiber filter (Whatman® 934-AH, GE Healthcare, Chicago, IL, U.S.). A 15.0 mL of second-filtered filtration was passed through an AflaTest® column at a rate of about one drop per second. The column was then washed by passing 20.0 mL of DI water at the same rate. The AFB1 binding on

the column was eluted by passing 1.0 mL of methanol. The eluate was collected in a test tube and sealed with Parafilm® (Bemis Company, Neenah, WI, U.S.) until fluorescence quantification. The bromine developer was prepared by diluting the bromine stock solution ten times in the dark before use. The diluted developer solution is sensitive to light and needs to be stored in an amber glass container for no longer than 2 h. The eluate was mixed with 1.0 mL of the diluted developer and fully vortexed. After 1 minute, the mixture was transferred to a 1.5 mL quartz cuvette and loaded into a Cary Eclipse Fluorescence Spectrophotometer® (Agilent, Santa Clara, CA, U.S.). The fluorescent absorption value was obtained by taking the average of 3 readings from the same eluate. The measured AFB1 concentration was calculated according to the AFB1 fluorescent absorption calibration curve (0 to 400.0 ng/g). The actual AFB1 concentration of the peanut sample was adjusted by the following formula:

$$c_a = (c_m/r) \times (25/w) \quad (4)$$

where c_a = actual AFB1 concentration (ng/g), c_m = measured AFB1 concentration (ng/g), r = column recovery rate (in this study, 0.80), w = peanuts weight (g).

In this article, the reduction percentage refers to the percentage of reduced AFB1, whereas the degradation rate refers to the velocity of AFB1 degradation. The calculation of AFB1 reduction percentage is listed below:

$$\text{AFB1 reduction percentage (\%)} = (c_o\text{-}c_r)/c_o \times 100\% \quad (5)$$

where c_o = original AFB1 concentration (ng/g), c_r = remained AFB1 concentration (ng/g) after treatment.

The calculation of AFB1 degradation rate is listed below:

$$\text{AFB1 degradation rate (pmol}\cdot g^{-1}h^{-1}) = (c_o\text{-}c_r)/(\text{M.W.} \times t) \quad (6)$$

where M.W. = molecular weight of AFB1 (in this study, 312.28), t = treatment time (hr).

Statistical analysis

The UV dosage profile was analyzed by linear regression (least-squares estimation) on Excel® (version 16.35, Microsoft, CA, U.S.). PCA, one-way ANOVA, and Tukey HSD mean comparison were performed on JMP® (version 12.4, SAS Institute, Cary, NC, U.S.).

Results and discussion

Properties of UV light source

To determine the warm-up time for the UV lamps we used, the intensity change with time was recorded (Figure 4.3A). As can be seen, the UV intensity was time-varying in the first 10 min. After 10 min, the UV intensity became more stable and slightly fluctuated within about ± 5%. The fluctuation could be due to the slightly varying ambient temperature near the lamps and can be ignored. All experiments in this study were started after a 10-min warm-up. Figures 4.3B and 4.3C show the UV intensity at different locations under a single UVC lamp. The UV intensity can be calculated by integrating irradiance from infinitesimal UV point sources along the length of the UV lamp and therefore is a function of distance following the inverse-square law. An about 40% intensity reduction can be observed at a location away from the center and 15 cm under the UV lamp, although the location was still covered by the range of the UV lamp (length of the lamp is about 34 cm).

UV dosage profile

The UV dosage profile was constructed by correlating L* and UV dosage. The color of AgCl emulsion can be described by three variables, L*, a*, and b*, under CIELAB color space. Although we expected only L* would be affected by AgCl, we found that a* and b* also changed after UV exposure. The change in a* and b* values could be due to the influence of background

color from peanut skin (Figure 4.4). A thicker emulsion coating could eliminate background color influence.

To simplify the calculation process, we introduced PCA for determining the most representative variable as the dosage indicator. According to the result (Table 4.1), only L* was chosen as the UV dosage indicator even though the changes in L*, a*, and b* were all proportional to the change in UV dosage. The eigenvalue of the PCA indicated that one variable contributed 99.7% of the variation, which should be adequate for representing the color change, although using the number of variables with an eigenvalue greater than 1 is recommended according to the Kaiser criterion. The eigenvector and partial contribution outcomes showed that the L* value was more sensitive to the color change on peanuts. Moreover, L* value demonstrated a better linear fit (R^2 = .87) than a* value (R^2 = .77) and b* value (R^2 = .47) under 0.5 mW/cm^2 UV irradiation. The relatively large standard deviations of L*, a*, b* were due to the influence of the background color from peanuts. The variation of the background color can be observed at zero seconds in Figure 4.5. A model based on more replicates should mitigate the issue. Although a* and b* values did not show an acceptable linear fit, constructing a non-linear model such as neural network classification involving three variables could assist in obtaining a better model fitting. The UV dosage measurement can be done by applying image processing technology to a photo, which has been performed to evaluate the temperature distribution of microwaved foods (Deng et al., 2003).

The gradient of L* value (slope of the linear equation) was greater under 1.0 mW/cm^2 UV irradiation than under 0.5 mW/cm^2. The difference met our expectation that the color change should be proportional to the change in UV dosage. We expected the slope should be 2-fold as UV intensity doubled. However, only a 1.6 times increase in the slope was observed (Figure 4.5). We found that even though the peanuts were coated with photography emulsion, the background color

of the peanuts can considerably affect the color finally exhibited, which can be evaluated by the standard deviation shown in Figure 4.5. The treatment size under 1.0 mW/cm^2 UV irradiation was less than that under 0.5 mW/cm^2, which could further augment the variation of peanuts. The treatment size for 1.0 mW/cm^2 UV irradiation was designed for equalizing UV dosage. Peanuts irradiated by 0.5 mW/cm^2 UV for 240 seconds had the same UV dosage as irradiated by 1.0 mW/cm^2 UV for 120 seconds: the theoretical UV dosages were both 120 mJ/cm^2. Adjusting the photography procedure or increasing sample size could mitigate the flaw. For example, thickening the photography emulsion coating could lower the influence of the background color of peanuts. The results showed that this profile was doubtlessly feasible for evaluating UV dosage distributed on solid foods.

UV uniformity improvement by rotating peanuts

The results showed that rotating peanuts improved UV uniformity. Without rotating, the variance of the L* value significantly increased as the UV irradiating time increased (Figure 4.6A), indicating that the UV dosage received on each peanut surface was not uniform. With rotating at different speeds, the improvement of UV irradiation uniformity showed varied trends despite no significant difference. When the peanut was rotated at 2 rpm, the trend of the variance of the L* value slightly increased but not as much as the peanuts without rotation. By visual observation, we found that about half of the peanuts slipped rather than being rotated on the surface of the stainless-steel chamber when rotation speed was 2 rpm, resulting in increasing non-uniform UV dosage distribution. When rotation speed was 11 or 20 rpm, we found that almost all the peanuts were well rotated. The observation was consistent with the data shown in Figure 4.6. Nonetheless, we found that peanut skins were damaged by the rotation. When peanuts were rotated faster than 11 rpm, the equipment worked like a peeling machine and caused the peanut to be peeled or even

split. A higher average of L* value was observed for the peanuts rotated at 20 rpm due to the damage of peanut skins as well as the emulsion coating.

The average L* value demonstrated that without rotating, rotating peanuts at 2, and 11 rpm did not have a significant effect on receiving UV irradiation (Figure 4.6B). The greater average of L* value of peanuts rotated at 20 rpm was due to the exposure of inside kernels, which was in yellow to white color. The damage of the skins was also observed on peanuts from the 11 rpm treatment, but the extent of the damage was negligible. Peanuts without rotation and those rotated at 2 and 11 rpm for the same treatment time had the same average of L* value level. As the treatment time prolonged, the average L* value became significantly lower than that for control.

The UV dosage delivered to each peanut can be obtained from the * value by the linear regression formula in the UV dosage profile (Figure 4.5). After 15-minute irradiation, the theoretical UV dosage should be 450 mJ/cm^2, whereas the observed dosages of the treatment without rotation, rotated at 2, and 11 rpm were 146 mJ/cm^2, 148 mJ/cm^2, and 158 mJ/cm^2, respectively. The difference between the observed and theoretical dosages comes from the topological issue in the color measuring process. The theoretical dosage is a sum of UV dosage received at the same sampling point on a flat surface in 15 min. The observed dosage, however, is an average sum of UV dosage received at four sampling points on the whole peanut surface in 15 min. The latter can be considered as a four-time wider area irradiated by the same UV irradiance and thus “diluted” the UV dosage. In addition, because the aperture of the colorimeter we used has a diameter wider than 1/4 periphery of a peanut, part of the sampling area would overlap with others. Given that, the total dosage received by each peanut cannot be simply multiplied by 4. The exact dosage received by a peanut should be calculated by more sampling points on the peanut with a narrower aperture.

On the other hand, we found that the weight of peanuts loaded in the stainless-steel chamber was able to affect the UV uniformity. The variance of L* values of loading 100 g, 200 g, and 300 g of peanuts in the chamber did not demonstrate a significant difference (Figure 4.7). However, the p-values of the pairwise comparison were closed to the significant level: p = .0547 between 100 g and 200 g; p = .177 between 100 g and 300 g. By visual observation, we noticed that as the weight of loaded peanuts increased, a multilayer stack of peanuts was formed and shaded the UV irradiation. Also, a heavier loading increased the friction between peanuts, blocking the rotation or movement of peanuts. The UV dosage received by peanuts became lower as more peanuts were loaded. The observed UV dosages for loading 100 g, 200 g, and 300 g of peanuts were 158 mJ/cm^2, 49 mJ/cm^2, and 93 mJ/cm^2, respectively. The p-value of 100 g-300 g pair comparison was 0.1004, which is close to the significant level. These results demonstrate that the peanut loading weight had an upper limit restricted by the dimensions of the container used for rotation. Excessive loading of peanuts will cause insufficient disinfection.

To sum up, the parameters for leveling UV dosage distribution in this study were determined. The loading weight of peanuts should be 100 g, and the rotation speed should be 11 rpm. The influence of leveled UV uniformity on the AFB1 reduction percentage was then examined.

Influence of UV uniformity improvement on AFB1 reduction percentage

Removing skin from peanuts significantly increased the AFB1 reduction percentage by UV irradiation. Our preliminary experiment showed that the AFB1 reduction percentages of peanuts with and without skins were 2.8 ± 0.9% and 31.8 ± 11.6%, respectively, and the AFB1 degradation rates of peanuts with and without skins were 4.6 ± 1.5 pmol·g^{-1}h^{-1} and 74.8 ± 27.1 pmol·g^{-1}h^{-1}, respectively (2.3 mW/cm^2 of UVC, 4 hours, n = 2). Almost no AFB1 reduction was observed if

the peanuts had the skin on, conflicting with the results that significant AFB1 reductions were observed in the previous studies (Garg et al., 2013; Jubeen et al., 2012). The effect of removing peanuts skin on AFB1 detoxification by UV was unexpected. Previous studies mostly focused on adjusting parameters such as UV intensity or treatment time for AFB1 detoxification. Instead, the inherent characteristics of food were of less interest.

The skin of the peanut seemed to have an ability to protect AFB1 from UV, according to our results. We thought that the AFB1 was degraded by indirect UV-induced free radical attack and direct UV photons strike. AFB1 is mainly distributed near the abaxial surface of the kernel under the skin (Cucullu et al., 1966). The skin could protect AFB1 from UV by both degradation pathways. The polyphenols in peanut skin may absorb UV-induced free radicals from the moisture or the cellulose and hence protect the AFB1 (Chukwumah et al., 2009; Nakamura et al., 1985). On the other hand, our results revealed that the skin was able to obstruct the penetration of UV irradiation. Further, in theory, the thickness of the peanut skin should block UV irradiation produced by most commercially available UV lamps, even a high-power medium-pressure UV lamp. Accordingly, the peeled peanuts were used for examining the effect of UV uniformity on AFB1 detoxification experiments.

The results demonstrated that leveling UV uniformity increased the AFB1 reduction percentage. After a 2-hour UV irradiation, we found that the AFB1 reduction percentage increased by 23.4%, from 14.3 ± 3.4% to 17.7 ± 4.5% (p = .3603), and the degradation rate increased from 60.8 ± 15.3 pmol·$g^{-1}h^{-1}$ to 75.0 ± 10.9 pmol·$g^{-1}h^{-1}$. Although the increase was not significant, all replicates showed an increasing trend of AFB1 reduction in the triplicate experiments. The skins that remained on hollows of peanuts could protect AFB1 from being fully exposed by UV. The AFB1 spiking process could also contribute to the result. The standard deviation of the AFB1

concentration observed in the same batch of spiked peanuts was 7.8% (n = 3). Both of the reasons could interfere with the final results. Increasing sample size or prolonging UV treatment time should magnify the effect of leveling UV uniformity on AFB1 detoxification. Overall, the result was consistent with the previous study, in which they confirmed that the UV disinfection rate could be increased by reflecting UV to the treatment subject from different angles (Xie & Hung, 2020).

Conclusions

The results from this study demonstrated that leveling UV dosage distribution can assist in enhancing disinfection efficiency. Rotating AFB1 contaminated peanuts increased the UV exposure of AFB1 and hence improved the AFB1 reduction percentage. Previous studies focusing on disinfecting solid foods by UV hypothesized that the UV uniformity could affect the disinfection rate. They provided the evidence to indirectly examine the hypothesis, whereas our work directly demonstrated the influence of UV uniformity on the disinfection rate.

For scaling up the process, more parameters such as dimensions of peanuts, or the frictions between peanuts and chamber should be considered. Additional rotation speed levels and loading weights can help understand the pattern of UV uniformity in a disinfection process. The proposed method performed appropriately but needed more improvement. The wavelength-independent quantum yields and sensitivity of AgCl should be further studied. Introducing a non-linear model can construct a UV dosage profile more efficiently. Reducing time-consuming tasks such as replacing manual color measurement with automatic imaging processing technology should also be considered. Additionally, investigation of applying the proposed irradiation uniformity evaluation method to other types of solid food such as powder is also required.

Acknowledgment

We appreciated the assistance of experiment works from Yuri Eduardo Pasian and Prateek Attry during their internships in the Department of Food Science & Technology at the University of Georgia.

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Tables

Table 4.1 Principle Component Analysis result of UV radiation profile.

Eigenvalues of unscaled matrix			
Number of variables	**Eigenvalue**	**%**	**Cumulative %**
1	3054.71	99.71	99.71
2	7.71	0.25	99.96
3	1.24	0.04	100.00
Eigenvectors			
Variable	**PC1**	**PC2**	**PC3**
L*	0.94	-0.33	-0.05
a*	0.21	0.47	0.86
b*	0.26	0.82	-0.51
Partial contribution of variables			
Variable	**PC1**	**PC2**	**PC3**
L*	89.14%	10.64%	0.22%
a*	4.20%	22.07%	73.73%
b*	6.66%	67.29%	26.05%

Figures

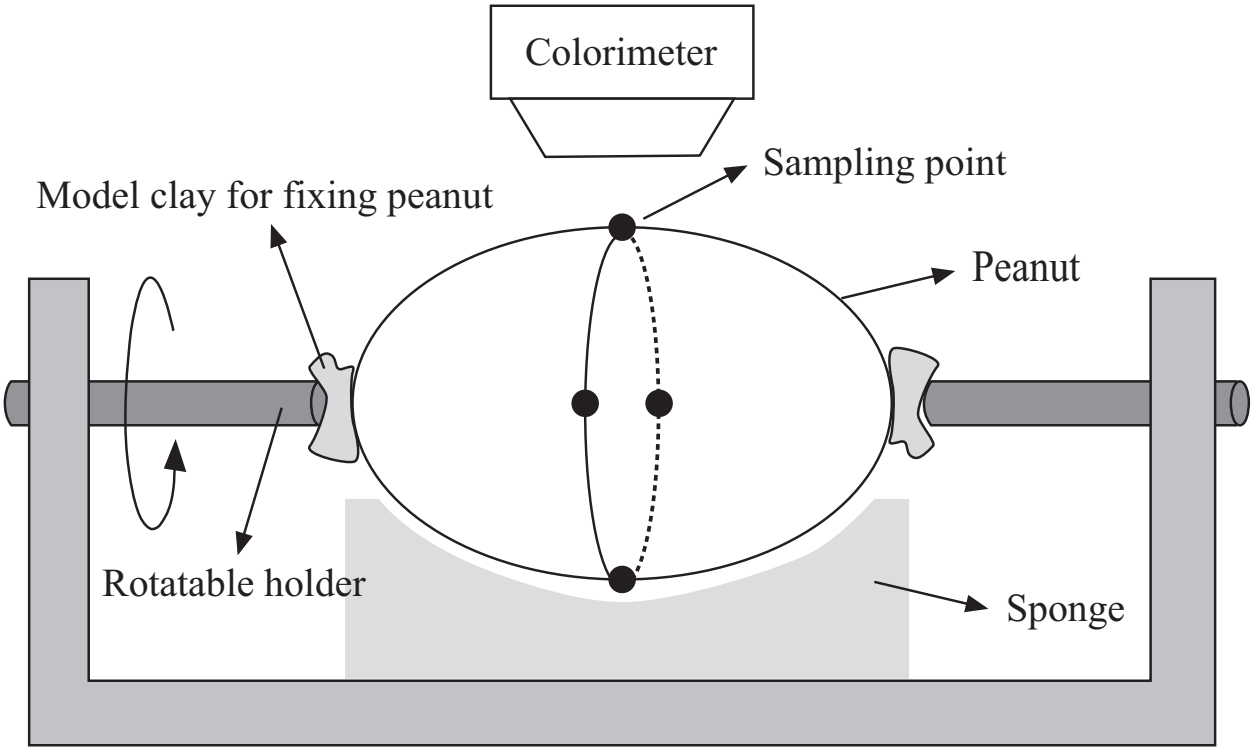

Figure 4.1 Diagram for measuring color on a peanut kernel.

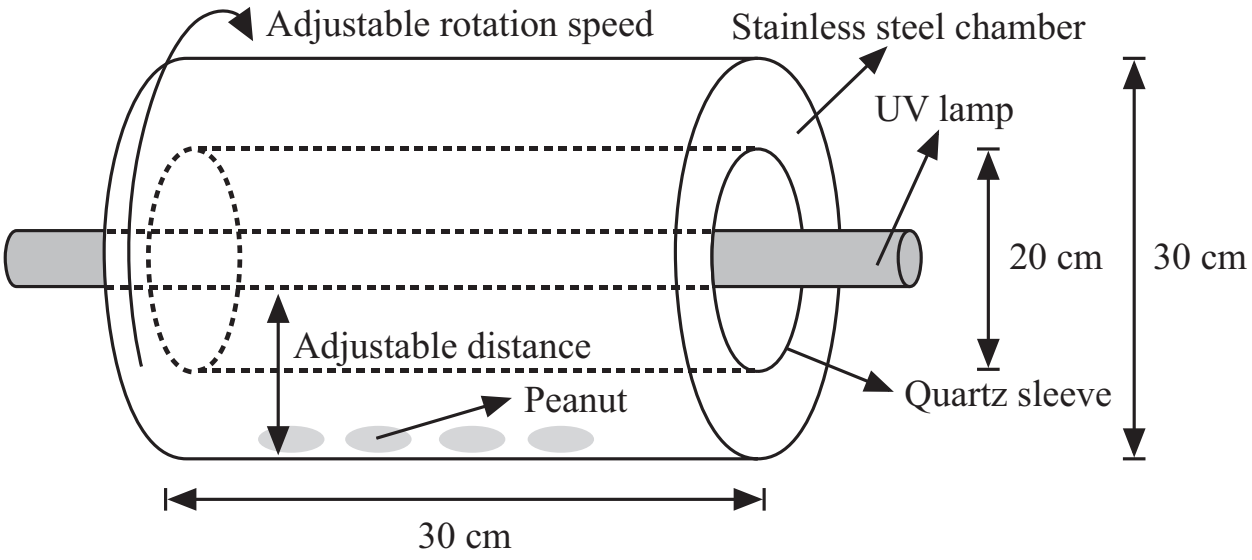

Figure 4.2 Schematic diagram of the UV treatment chamber.

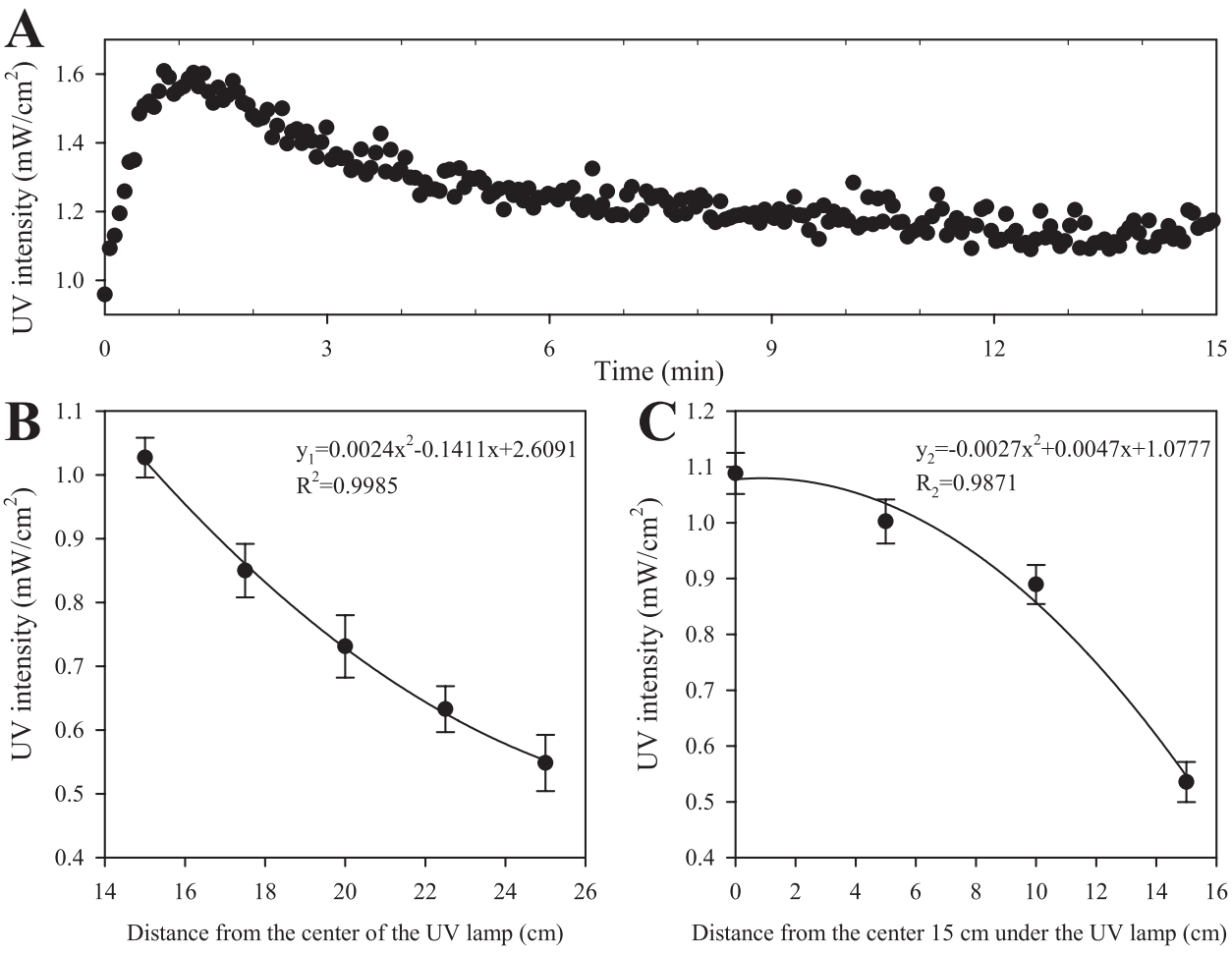

Figure 4.3 Intensities of UV irradiation changed by (A) run time of the UV lamp (B) distance from the center of the UV lamp (C) distance from the center 15 cm under the UV lamp.

Figure 4.4 Color changes of AgCl emulsion coated peanuts after exposure to UV at an intensity of 1 mW/cm^2 for 0, 40, 80, and 120 s (left to right).

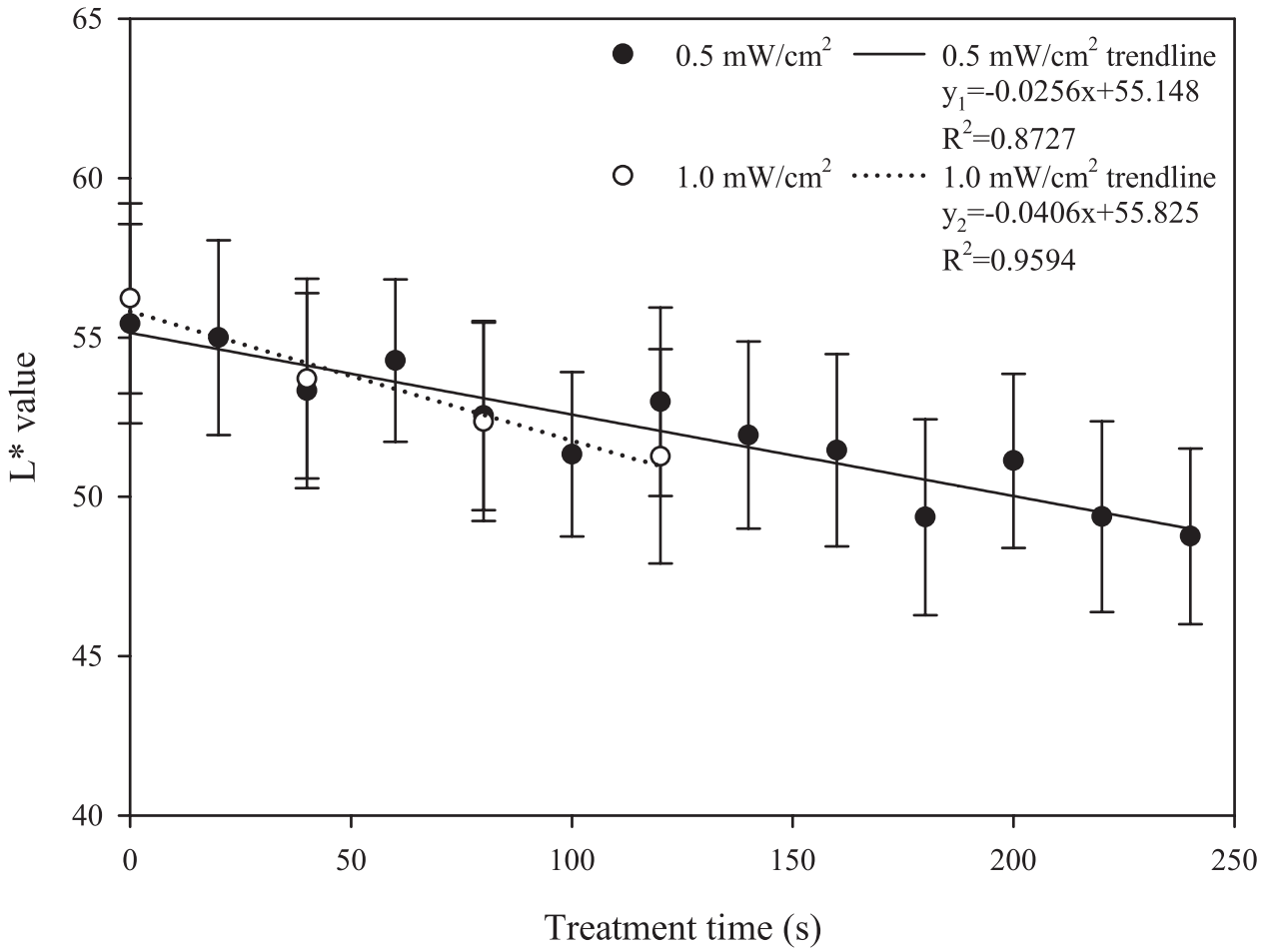

Figure 4.5 UV dosage profile based on L* value. UV dosage (mJ/cm^2) can be obtained by multiplying treatment time (seconds) and intensity (mW/cm^2).

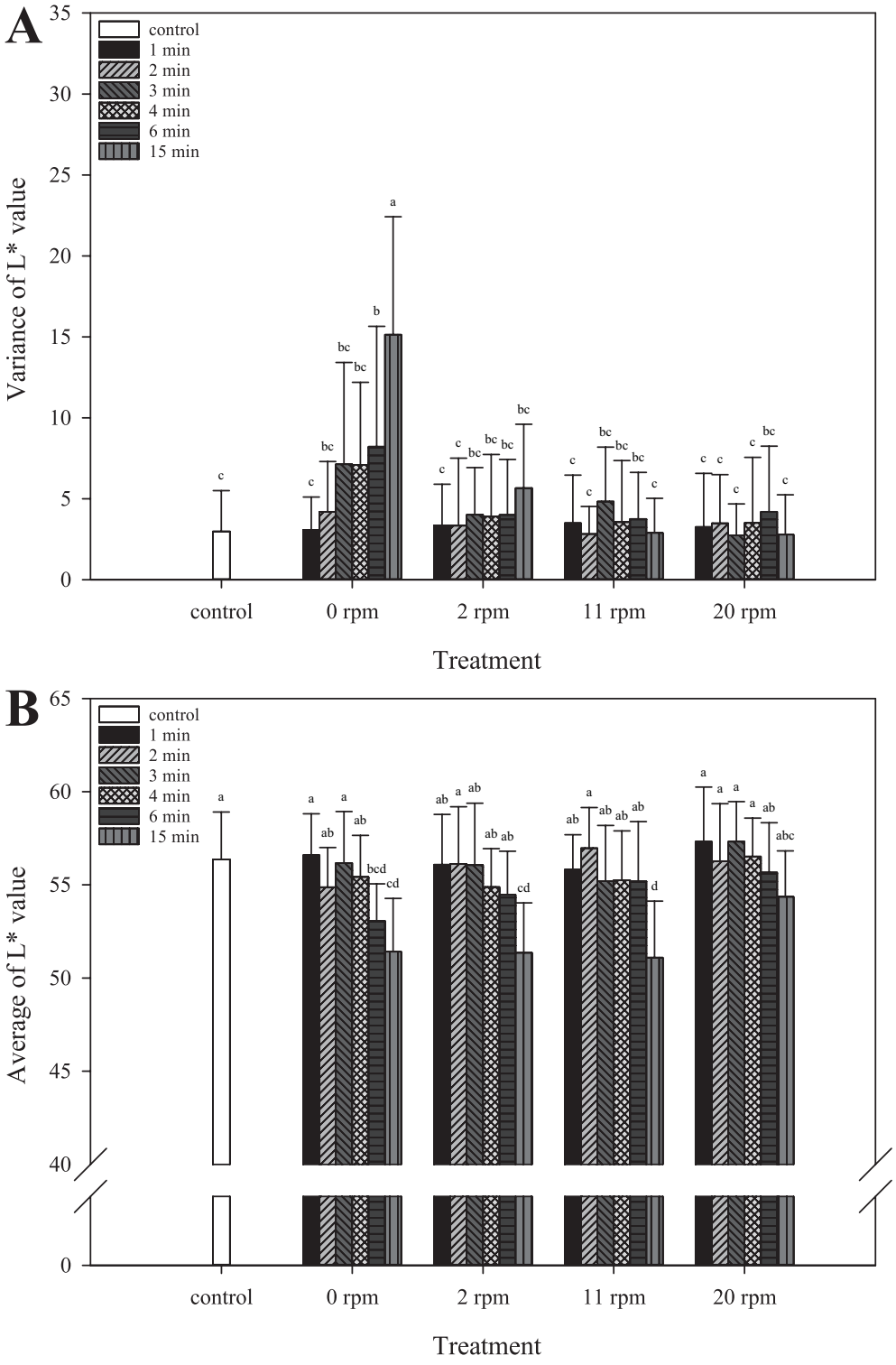

Figure 4.6 The (A) variance of L* values and (B) average of L* values of peanuts irradiated by UV and rotated at 2 rpm, 11 rpm, 20 rpm, and without rotation for 1-15 minutes ($p < .05$).

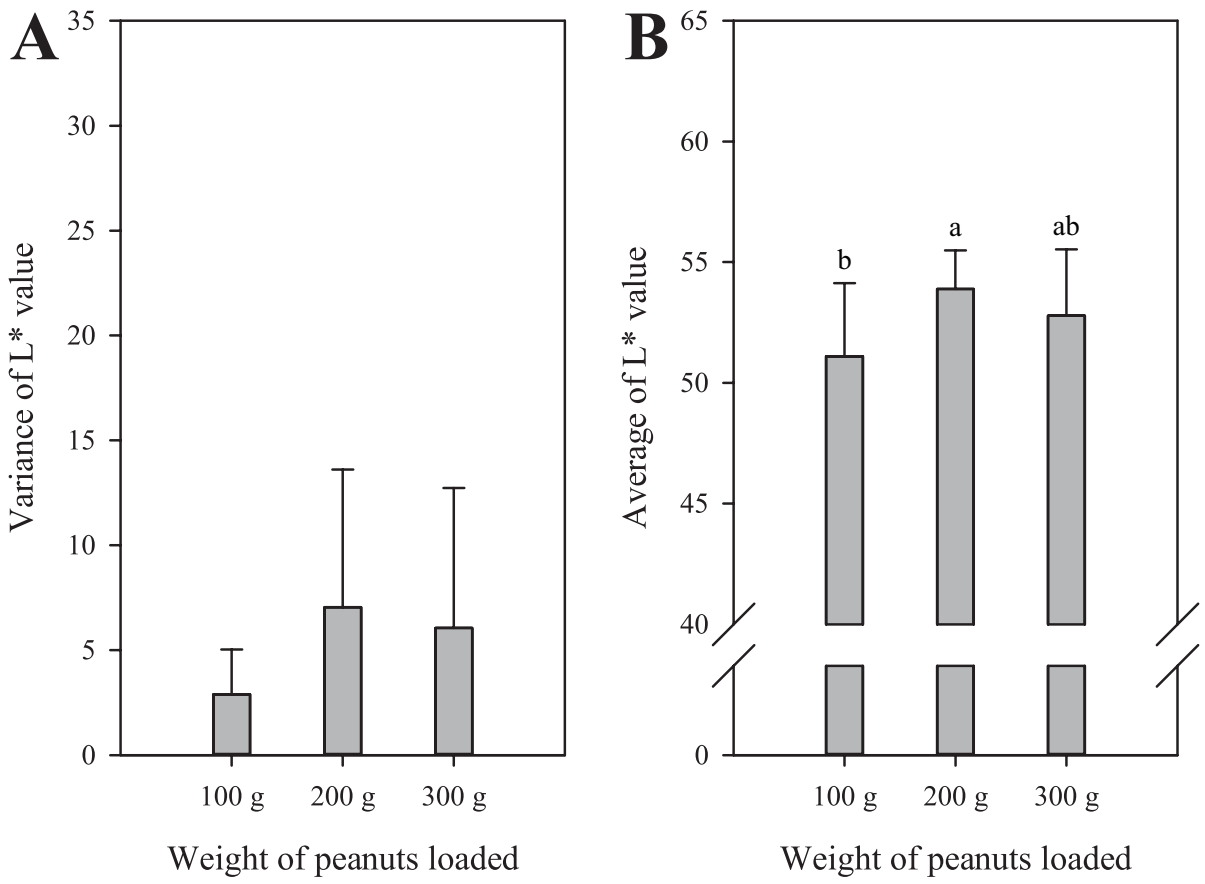

Figure 4.7 Influence of loading peanut weight on (A) variance of L* value and (B) average of L* value ($p < .05$).

CHAPTER 5

EFFECTIVE UV WAVELENGTH RANGE FOR INCREASING AFLATOXINS REDUCTION AND DECREASING OIL DETERIORATION IN CONTAMINATED PEANUTS[1]

[1] Shen, M. H., & Singh, R. K. 2022. *Food Research International*. 154, 111016. Reprinted here with permission of the publisher.

Abstract

Many studies have demonstrated that UV radiation can degrade aflatoxins (AF) in contaminated foods. However, the effective wavelength ranges for AF decomposition and their impacts on the quality of foods have not been elucidated. This study investigated the AF reduction and oil quality change in peanuts subjected to three types of 17 W low-pressure (LP) UV lamps covering UV-A (Max. emission: 365 nm), UV-B (Max. emission: 310 nm), and UV-C (Max. emission: 254 nm) ranges and a 2000 W medium-pressure UV (MP) lamp covering from UV-A to UV-C. We used peeled kernels for this study since the peanut skin represented an ability to protect AF from being degraded by UV. LP UV-A lamp treatment has shown the highest AF reduction in artificially spiked peeled kernels and no detectable oil deterioration. With the same delivered UV dosage as LP lamp, MP lamp has shown the same level of AF reduction as LP UV-A lamp did, indicating such treatment was energy inefficient. Treating *Aspergillus nomius* inoculated peeled kernels by two LP UV-A lamps (2.76 mW/cm^2) for 1.0 h reduced 40% of AF if the kernels were milled into 1 mm-diameter particle, implying that exposing the interior part of kernels to UV radiation is necessary for an AF decontamination process. In the oil deterioration test, we found that UV-C strongly induced the oil oxidation of peanuts. Accordingly, we concluded that UV-A is the effective wavelength range to degrade AF as well as maintain the oil quality in foods allowing UV radiation to well penetrate the sample, such as liquid foods with low turbidity or solid foods in the particle form. These results also justify the use of solar radiation as an AF decontamination method, rendering this method to be employed in areas that lack infrastructure.

Introduction

Aflatoxins (AF) are a group of mycotoxins produced by mold species, such as *Aspergillus flavus* and *A. parasiticus*, and can cause many adverse health effects (Gong et al., 2016; Wu et al., 2008). Consumption of AF contaminated foods can lead to failure of the human liver and kidney, as well as the impairment of the immune system and the growth of children (Chen et al., 2018; Mousavi Khaneghah et al., 2018; Turner et al., 2003). Additionally, one of AF derivatives, aflatoxin B1, has been recognized as the class 1 carcinogen by IARC (2022), which is a major public health issue in developing countries. Aside from humans, poultry and livestock are also susceptible to AF after consuming contaminated foods or feeds. Feeding animals with AF-contaminated crops can result in health issues similar to that of effects on the human, including liver damage, immunosuppression, and lower production (Atherstone et al., 2016). The products from those animals fed with contaminated commodities, such as meats and milk, may also become contaminated with AF or its derivatives (Atherstone et al., 2016). Accordingly, authorities around the world have regulated the concentration of AF in not only human foods but also in animal feeds. For example, the US FDA (2018) has set different AF action levels for commodities for different purposes, e.g., 20 ng/g for human foods and 100 to 300 ng/g for animal feeds. The AF contamination hence causes considerable economic impacts from either the discarded of contaminated foods or the management of the contamination (Wu et al., 2008).

Many attempts for the amelioration of AF-contaminated crops have been made by applying UV irradiation, which possesses characteristics such as low cost, high efficiency, and low-quality impacts on foods (Shen & Singh, 2021a). UV processing has been successful in reducing not only AF concentration but also the toxicity of decomposed AF compounds in treated foods (Diao et al., 2015; Mao et al., 2016; Shen & Singh, 2021a; Stanley et al., 2020). However, the ranges of UV

wavelength employed in this type of study were varied, although they all showed a significant AF reduction. Mao et al. (2016), Stanley et al. (2020), and Tripathi & Mishra, (2010) applied UV sources that emitted radiation at 365 nm, whereas Biosci et al. (2016), Garg et al. (2013), and Li et al. (2019) applied UV sources that emitted radiation at 254 nm. Both wavelength ranges can successfully decontaminate AF. Stanley et al. (2020) explained that AF has a maximum absorption at about 365 nm, which is covered in the UV-A range and can act as photosensitizers to form reactive oxygen species (ROS). These ROS then react with AF and result in AF degradation. Based on their theory, UV sources with a maximum wavelength emission at 254 nm, which is covered in the UV-C range, should be able to generate more ROS and therefore induce more AF reduction. This is because many molecules in the food matrix such as biomolecules or water can work as photosensitizers when absorbing radiation in the UV-C range (Michaelian, 2021; Widel et al., 2014).

On the other hand, oil deterioration is a concern when employing UV radiation for processing oily foods like peanuts. UV can induce photooxidation of oil by producing ROS, leading to the production of free fatty acids, aldehydes, and ketones, and eventually causing oil deterioration (Pascall et al., 1995). Nonetheless, Garg et al. (2013) and Shen et al. (2014) indicated that either treating peanuts with 254 nm radiation or treating peanut oil with 365 nm radiation did not significantly cause oil deterioration of the sample. Both studies used several indicators, such as peroxide value, *p*-anisidine value, or acid value, for evaluating oil quality change after UV treatments, but these indicators showed only concentrations of certain oxidative products at certain stages in an oil deterioration process, which hence may neglect the effect of UV on the long-term quality change in oil (Zhang et al., 2021).

The effect of applying a single UV wavelength range on AF reduction in a variety of foods has been well studied. However, comprehensive knowledge regarding the effect of different UV wavelengths on AF degradation and food quality is still unclear. Accordingly, we aimed at investigating the effect of different UV wavelength ranges on reductions of four AF (B_1, B_2, G_1, G_2) reductions and oil quality in fresh shelled peanuts. This study has four specific objectives: (1) to compare the AF degradation in peanut-peel and peeled kernels under UV radiation, (2) to determine the effective UV wavelength range for degrading AF in artificially AF-contaminated whole peanut kernels, (3) to evaluate the oil quality changes after UV treatment, and (4) to verify the AF degradation by determined UV wavelength range on *Aspergillus* contaminated peanuts.

Material and Methods

Chemicals

Aflatoxin B_1 (AFB_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1), Aflatoxin G_2 (AFG_2), and NaCl, were ACS grade and purchased from Sigma-Aldrich (MO, USA). Methanol (HPLC grade), formic acid (HPLC grade), and Tween 80 were purchased from Fisher Chemical (NJ, USA). $MgSO_4$ was ACS grade and purchased from J. T. Baker Chemical (NJ, USA). Toluene, ethyl acetate, and acetonitrile were HPLC grade and purchased from Sigma-Aldrich (MO, USA). Potato dextrose agar (PDA) was purchased from Millipore (Darmstadt, DE).

AF stock solution preparation and absorption curves

AF stock solutions were prepared by dissolving AF crystals in methanol to form about 10 μg/mL AF solutions according to the AOAC official method 971.22 (AOAC, 2000). AF stock solutions were stored at 4˚C in the dark. The absorption curves of four AF in the wavelength range from 200 to 500 nm with a resolution of 1 nm were recorded by a UV-Vis spectrophotometer (Model UV-1601, Shimadzu, Kyoto, Japan).

UV lamps and equipment

To find a specific wavelength and the corresponding light source for efficiently decomposing AF is impractical. Therefore, we selected 3 commercially available low-pressure (LP) mercury UV lamps (17 W, Model GPH357T5L/4P, LightSources Inc., CT, USA) approximately covering UV-A, UV-B, and UV-C range and a customized 2000 W medium-pressure (MP) mercury UV lamp (LightSources Inc., CT, USA) covering a continuous spectrum from UV-C to infrared. The specific properties of the lamps are shown in Table 5.1. The UV-C lamp emits monochromatic radiation with a wavelength at around 254 nm generated by excited mercury atoms, whereas the UV-A and UV-B lamps emit broader spectrums at around 365 nm and 310 nm, respectively, from the coated phosphors on the lamp tubes excited by UV-C (Bolton & Linden, 2003). To power LP lamps, an 18 W power supply (Model PSM2GPH18MVDW, Robertson, Blue Island, IL, USA) was used. For the MP lamp, a 3-kW lamp driver (Nedap Inc., DC Groenlo, NL) was used. The irradiances from UV lamps were measured by a radiometer, (Model ILT800-UV, CureRight, MA, USA).

Evaluation of the AF degradation in peanut-peel and peeled-kernels

The role of peanut skin in protecting AF from being degraded by UV was evaluated for improving detoxification efficiency in our previous work (Shen & Singh, 2021b). Fresh and shelled peanuts (runner type, *Arachis hypogaea* L.) with less than 5 ng/g of AF were obtained from a local manufacturer, Golden Peanut Corporation (GA, USA). Peanuts were then vacuum-sealed and stored at 4˚C before being used. Peanuts were blanched by an impingement oven (Model 1450, Lincoln, Fort Wayne, IN, USA) at 100˚C for 10 min followed by a customized peeling machine described in Kettler et al. (2017). The peeled kernels and peanut peel were separately collected and milled into about 1 mm-diameter particle by a blender (Model WF2211214, Waring

corporation, CT, USA). The milled peeled kernels and peanut peel were spiked with AF stock solutions to obtain samples containing 50.0 ng/g AFB_1, 12.5 ng/g AFB_2, 25.0 ng/g AFG_1, and 12.5 ng/g AFG_2 by the method described in Shen & Singh (2021b). Due to the density difference, 7.5 g of the spiked peeled-kernels and 1.5 g of the spiked peanut-peel were spread as one layer thick in Petri dishes as shown in Fig. 5.1 (Pyrex 100 mm × 20 mm, Corning, Glendale, AZ, USA), ensuring that the exposure-area of the two types of samples were the same. The Petri dish loaded with the spiked sample was subjected to 2.0 h of 2.18 mW/cm^2 UV radiation from two UV-C lamps combined with reflectors held at 10 cm high above the sample. The concentrations of AF were determined by AF assay. The experiment was performed in duplicate.

Determination of effective UV wavelength range for degrading AF

To eliminate the influence of peanut skin on AF degradation by UV, whole peeled kernels were prepared to contain 100.0 ng/g AFB_1, 25.0 ng/g AFB_2, 50.0 ng/g AFG_1, and 25.0 ng/g AFG_2 as described in Section "Evaluation of the AF degradation in peanut-peel and peeled-kernels." To evenly expose whole peeled kernels to UV irradiation, the equipment described by Shen & Singh (2021b) was used. Four UV lamps shown in Table 5.1 were used to treat the samples. Two LP lamps or one MP lamp were held at 10-cm high above the sample, delivering 2.76 mW/cm^2 (using LP UV-C lamps) or 12.48 mW/cm^2 of UV radiation, respectively. For samples subjected to LP lamps, 150 g of spiked peeled kernels were loaded in the treatment cylinder and rotated at 11 rpm for 10 s followed by a stoppage of rotation for 350 s. The process was repeated 10 times (or 1 h). At thirtieth and sixtieth min, 25 g of peeled kernels were sampled and analyzed. For samples subjected to MP lamps, the same amount of spiked peeled kernel was loaded and rotated at 11 rpm but only for 10 s followed by a stoppage for 50 s. The process was repeated 10 times (or 10 min). At the fifth and tenth min, 25 g of peeled kernels were sampled and analyzed. To deliver UV

dosages at the same order of magnitude, the processing time of the MP lamp treatment was shortened to 10 min. The experiment was performed in duplicate.

Oil quality evaluation using differential scanning calorimetry (DSC)

The oil quality was determined by comparing the oxidation induction time (OIT) of the UV treated sample with the control. This method has a high correlation with total oxidation value (TOTOX) and is very sensitive (Hyatt et al., 2021; Zhang et al., 2021). The milled peeled kernel (7.5 g) was prepared, loaded in a petri dish (Pyrex 100 mm × 20 mm, Corning, Glendale, AZ, USA), and treated with LP UV-A and LP UV-C lamps as described in Section "Evaluation of the AF degradation in peanut-peel and peeled-kernels" for 1.0 h. The treated sample was collected from the petri dish and subjected to a cold pressor (Model 3853, Carver Inc., IN, USA) to obtain oil for OIT analysis. To further investigate the effect of UV wavelength ranges on oil quality, 1 g of the cold-pressed oil from fresh whole peeled kernel was loaded in a petri dish (Pyrex 55 mm × 17 mm, Corning, Glendale, AZ, US) followed by treating with LP UV-A and LP UV-C lamps as described in Section "Evaluation of the AF degradation in peanut-peel and peeled-kernels" for 15 min. The treated oil was collected for OIT analysis.

The oil samples (20.0 ± 0.5 mg) from each treatment were pipetted into 40 µL aluminum crucible pans (Model ME-27331, Mettler Toledo, OH, USA) without covering lids. Pans with oil samples were loaded in a DSC (Model DSC-1 700, Mettler Toledo, OH, USA) previously stabilized at 25˚C and purged with 50 mL/min of N_2. OIT analysis was performed by increasing temperature from 25˚C to 140˚C at a rate of 40˚C/min and then held for 3 min for stabilization. After stabilization, purging gas was switched to O_2 (50 mL/min) to start the oxidation process. The OIT was the time needed for the oil sample to oxidize, which was the start time of purging O_2 to the onset time of the exothermic reaction. The onset time was determined by calculating the

intersection point of the extrapolated baseline and the tangent line of the leading edge at the exothermic peak of the isotherm. All samples were performed in duplicate.

Verification of the proposed UV treatment by Aspergillus contaminated peanuts

The UV treatment was verified by using whole peeled kernels inoculated with NRRL 6108 (*Aspergillus nomius*). The peanut inoculation process was modified from Pennerman et al. (2019). About 0.1 g of lyophilized spore pellet obtained from Agricultural Research Service Culture Collection (IL, USA) was dissolved in 2 mL of sterile DI water to form spore suspension. After the pellet was completely dissolved, 0.1 mL of the suspension was transferred on PDA loaded in a petri dish (100 mm × 15 mm, VWR, Radnor, PA, USA) and then incubated at 30˚C, RH >90% for 4 days. The spore suspension was harvested by washing 10 mL of sterile DI water containing 1% of Tween 80 on the inoculated Petri dishes followed by being diluted to 10^6 spores/mL. A batch of 2 kg of whole peeled kernels prepared as described in Section "Evaluation of the AF degradation in peanut-peel and peeled kernels" was rinsed with 500 mL of sterile DI water and drained followed by mixing with 40 mL of the spore suspension in a food storage box (28 cm × 18 cm × 8 cm, Wal-Mart Stores Inc., AR, USA). Peeled kernels were continuously stirred until no liquid was observed. The food storage box was covered with surgical masks to allow respiration of fungi and then incubated at 30˚C, RH >90% for 6 days. The box was shaken to uniformly distribute the mycelia every 24 h during the incubation.

The inoculated peeled-kernels were treated by the UV equipment installed with two LP UV-A lamps and the same rotation parameters as described in Section "Determination of effective UV wavelength range for degrading AF" for 4.0 h. Sampling was done by withdrawing about 25 g of kernels from the UV equipment at the first hour and the fourth hour during UV processing.

Since a low AF reduction of the whole peeled kernels after UV-A treatment was observed, we milled the whole peeled kernels into about 1 mm-diameter particle. The particles were then placed in Petri dishes and subjected to LP UV-A irradiation as described in Section "Evaluation of the AF degradation in peanut peel and peeled kernels" for 1.0 h. The UV-treated samples were analyzed by AF assay. The experiment was performed in triplicate.

Aflatoxin assay using immunoaffinity column and HPLC-Fluorescence detector (HPLC-FLD)

The AF determination was done by modified AOAC method 991.31 (2002) and normal phase HPLC-FLD method (Leitao et al., 1988; Manabe et al., 1978). Peanuts samples were weighed and recorded to the second decimal of gram. The weighed sample was mixed with 125 mL water/methanol (30/70, mL/mL) and 5 g of NaCl. The mixture was blended by the blender mentioned in Section "Evaluation of the AF degradation in peanut-peel and peeled-kernels" at high speed for 2 min followed by filtering with a fluted filter paper (24 cm, Fisher Scientific, NH, USA). A 12 mL of filtrate was diluted with 20 mL DI water and then filtered with a 1.5 μm glass microfiber filter paper (Whatman® 934-AH, GE Healthcare, IL, USA). An AflaTest® P immunoaffinity column (Vicam, MA, USA) loaded with the secondary filtrate (10 mL for artificially spiked sample, 2 mL for fungi inoculated sample) was pumped (Vicam, MA, USA) with air to assist the filtrate to pass through the column at a rate of about one drop per second. The column was then washed with 20 mL of DI water at the same rate with the assistance of the air pump. A 0.45 mL of acetonitrile was pipetted into the column to elute AF. Acetonitrile was passed through the column by gravity. Pumping air by a syringe manually during elution might be needed if bubbles obstruct the column. At the end of elution, the column was pumped with 10 mL of air by a syringe manually to drain the remaining acetonitrile. The collected eluate was mixed with 0.2 g of $MgSO_4$ and 0.3 mL of toluene in an ice bath followed by being vortexed until the mixture

became clear. The eluate without $MgSO_4$ debris was then transferred to a 2.0 mL HPLC sample vial with a 0.4 mL insert (Fisher Scientific, NH, USA).

The HPLC analysis was performed on an Agilent 1260 Infinity HPLC system (CA, USA). The mobile phase was the mixture of two solutions, toluene/ethyl acetate/formic acid (45/3/2, mL/mL/mL) and toluene/ethyl acetate/methanol (45/3/2, mL/mL/mL), with the ratio of 50:50 (mL/mL) in the pump. The flow rate of the separation column (LC-Si, 5 μm, 25 cm × 4.6 mm, Supelco, PA, USA) was set at 1.5 mL/min under 30˚C. The sample injection volume was 40 μL. Premixing the mobile phase outside the pump will lead to spontaneous esterification between formic acid and methanol. Two solutions can be stored at room temperature in the dark for up to two weeks. The column was flushed with toluene/ethyl acetate (90/10, mL/mL) at a rate of 0.5 mL/min for 1.0 h before and after the analysis. The fluorescence detector was set to excite analytes at 365 nm and receive the fluorescence at 425 nm with a sampling rate of 2.31 Hz. The concentration was determined by integrating peak areas to obtain corresponding values. The final concentration of AF in the sample can be obtained by the following equations:

$$W = S\ g \times (12\ mL/125\ mL) \times (I\ mL/32\ mL) \quad (1)$$

$$E = E_{MeCN} + E_{Tol} = 0.75\ mL \quad (2)$$

$$\text{Aflatoxin in peanut (ng/g)} = (A \times E) / W \quad (3)$$

where W = equivalent weight of sample fo the eluate (g), S = weight of peanut sample (g), I = volume of the secondary filtrate passed through the immunoaffinity column (mL), E = volume of acetonitrile to elute aflatoxin from IAC and toluene to dilute the eluate (mL), and A = concentration of aflatoxin in eluate injected into HPLC (ng/mL). Total AF was the sum concentration of AFB_1, AFB_2, AFG_1, and AFG_2.

Statistical analysis

Statistical significance with a significant level, $p<0.05$, was determined by the ANOVA table and Tukey's pair test. The analysis was performed on JMP Pro 15.0.0 software (SAS Institute Inc., NC, USA).

Results and Discussion

AF degradation in peanut peel and peeled kernels

The results indicated that peanut skin can protect AFB_1, AFB_2, and AFG_1 from being decomposed by UV-C radiation (Table 5.2). The insignificant changes in AFG_1 after UV-C treatment in either milled kernel or peel could be due to the less affinity of the immunoaffinity column used in this study. The immunoaffinity column is insensitive to AFG_2 especially when the concentration is low, which was also observed in Trucksess et al. (1991), so the AFG_2 change after the treatment might be less noticeable. The significant decrease in AFB_1 was the most valuable information since the toxin represents the highest toxicity and its concentration is dominant in contaminated foods (Samarajeewa et al., 1990; Van Egmond & Jonker, 2004). The reduction of total AF was significant ($p<0.05$) and almost two-folds in peeled kernel than in peanut peel. In our previous work with UV application, we found that the peeling process considerably increased the AF reduction in whole kernels from about 3% (with skin on) to 32% (after being peeled)(Shen & Singh, 2021b). Accordingly, we can conclude that the peanuts peel can shade the kernels from UV radiation and hence avoid AF being exposed to the radiation. On the other hand, this study demonstrated another AF protection mechanism of the peanut skin. The skin seems to be able to protect the AF inside the skin from being degraded by UV radiation by the hypothesis proposed in Shen & Singh (2021b) that the polyphenols in the peel could function as free radical scavengers, absorbing radicals produced by the interaction between the UV and the peanut matrix

(Chukwumah et al., 2009; Nakamura et al., 1985). As a result, we used peeled kernel as the material in the following experiments.

Determination of effective UV wavelength range for degrading AF

In this study, only the intensity (irradiance) of LP UV-C lamps (Sections "Evaluation of the AF degradation in peanut-peel and peeled-kernels" and "Determination of effective UV wavelength range for degrading AF") was recorded. This is because the intensities of signal response at each wavelength from the sensor of the radiometer varied (InternationalLight Technologies, 2021). According to the datasheet of the sensor, the irradiance at 365 nm could be almost three times that at 254 nm. We measured 4.51 and 8.35 mW/cm^2 of irradiance from LP UV-B and LP UV-A lamps, respectively, but the only physical difference between the two LP lamps was the use of phosphors. Hence, we used the irradiance measured from LP UV-C lamps as the standard, based on the assumption that the energy conversion efficiencies of the phosphors used in those lamps are 100%. To estimate the irradiance of the MP lamp, we assumed that the measured value was the average of irradiances at each wavelength distributing from UV-C to UV-A (Cutler & Zimmerman, 2011).

Both LP UV-A and LP UV-C lamp treatments showed a significant reduction after 1.0 h of UV irradiation, but AF degradation patterns are different (Table 5.3). In the first 0.5 h, LP UV-A treatment shows a significantly higher total AF reduction than the other two types of LP lamps. After 1.0 h irradiation, LP UV-A treatment shows the highest reduction percentage. The AF reductions were almost "saturated" at the first 0.5 h under the UV-A radiation, revealing that the AF decomposition directly involved the reaction of AF molecules themselves. For instance, the electrons of AF were activated by absorbing radiation in the UV-A range. Fig. 5.2 demonstrates the absorption curves of AF stock solutions mentioned in Section "AF stock solution preparation

and absorption curves." The relative maximum absorption peaks of AF locate around 350 to 370 nm, which implies that AF could react as photosensitizers under the wavelength range of the LP UV-A lamp (Stanley et al., 2020). Nonetheless, those activated photosensitizers can directly react with water or other molecules and then trigger subsequent degradation processes without the participation of ROS. The total AF reduction significantly increased after 0.5 h irradiation, indicating that the pattern of AF decomposition under LP UV-C lamps was different from that under LP UV-A lamps. The delayed increase of AF reduction could be due to the accumulation of ROS produced in the peanut matrix, such as water, oxygen, or cellulose (Nakamura et al., 1985). In this pattern, AF reduction occurred only after ROS generated, so the ROS production and accumulation can be considered as a rate-determining step. It could be the reason why UV-B radiation neither activates AF as much as UV-A nor produces as many ROS as UV-C and thus the LP UV-B lamps demonstrated the lowest AF reduction.

Even though the MP lamp demonstrates a great ability to degrade AF in several minutes, it could not be an ideal method for decontaminating foods. In our observation, the temperature of peanuts subjected to the MP lamp increased to more than 90˚C in the first tenth min, whereas LP lamps increased the temperature to only about 30˚C after an hour of irradiation. Further, the MP lamp consumed almost 60 times higher energy but had the same level of AF reducing as the two LP lamps did. The wavelength distribution (Table 5.1) shows that a lot of energy was wasted on the radiation in the wavelength range from 400 to 1200 nm which is of much less interest. The costs for the equipment and replacing lamps are also an issue. The cost for the driver of two LP lamps was around $30, whereas the driver for a MP lamp cost more than $1000. The lifetime for LP lamps is 8000 h, which is much longer than 1000 h for an MP lamps. Accordingly, we recommend using LP UV-A and LP UV-C lamps for AF decontamination.

Oil quality evaluation using differential scanning calorimetry (DSC)

It seems like the peeled kernel can still protect the nutrients from being impaired by UV irradiation. In our observation, there was no significant change in peroxide value, *p*-anisidine value, or even OIT in whole peeled kernels treated by LP UV-C lamps (data not shown). This could be due to the limited penetrability of the UV radiation, so only the oil distributed on the surface was affected. However, we found that kernels treated with UV-C had a strong smell of oil deterioration, indicating the rancidity had occurred during the UV treatment. Hence, to accelerate the oil oxidation, we used a milled peeled kernel as the sample in the later test.

It is noticeable that LP UV-A lamps did not significantly lead to the quality change of oil in either milled peeled kernel or cold-pressed peanut oil (Table 5.4). UV radiation is recognized as a strong oxidation-inducing factor (Pascall et al., 1995). As can be seen, the peanut oil exposed to the UV-C for 15 min was oxidized much more quickly than the control. The OIT was shortened to 20%. Even with the protection of the peanut matrix in the milled kernel sample, the OIT was reduced to about 50%. The result indicates that UV-C is not suitable for AF decontamination, even though it demonstrated a great AF degradation ability. The oxidation issue is predictable when employing an MP lamp for decontamination due to the emission in the UV-C range (Cutler & Zimmerman, 2011), despite the lamp having a significant emission peak at 365 nm. Although UV-C is not appropriate for AF decontamination, the disinfection ability still can be used in preventing the growth of AF-producing fungi and the subsequent AF contamination (Udovicki et al., 2022).

These findings justified the use of UV-A in decontaminating liquid foods such as peanut oil or milk (Prandini et al., 2009; Shen et al., 2014). As aforementioned, UV-A does not have a significant impact on oil quality within an hour of irradiation. Additionally, there have been many available continuous UV photoreactors for processing liquid foods by UV. For those places without

sufficient infrastructure, decontaminating AF-contaminated liquid foods by solar irradiation should be a practical method to achieve the food safety requirement since the sun is an abundant source of UV-A (Marzo et al., 2018; Samarajeewa et al., 1985).

Verification of the UV treatment by Aspergillus contaminated peanuts

Preparing fungi inoculated peanuts with uniformly distributed AF is a challenge. As can be seen in Table 5.5, the control group has varied AF concentrations (33% of relative standard deviation for total AF) even though we made an effort to evenly grow the fungi on each kernel. The result accords with Martins et al. (2017), which represented 62% of the relative standard deviation of AF concentration. Due to the large standard deviation, it is difficult to conclude whether the reduction was contributed by the uneven distribution of AF or the UV-A radiation.

To further investigate, we milled the kernels and mixed them well to obtain samples with a more uniform AF distribution. The standard deviation of AF in the control group shows that the milling process largely lowers the variation of total AF concentration to 7% of relative standard deviation. Additionally, breaking down the whole peanuts into small particles seems more effective than improving the uniformity of UV radiation in a UV decontamination treatment. According to the analysis of AF distribution, most AF was located on or near the surface of peanuts (Cucullu et al., 1966; Goldblatt, 1966). However, the poor penetrability of UV radiation may not be able to reach the interior areas. The unchanged OIT of whole peeled kernels treated by UV-C demonstrates the difficulty of radiation to affect the oil in the deep area. On the other hand, as a type of phytotoxin, AF has evolved great mobility to migrate inside the plant tissues, increasing the difficulty to completely decompose AF by UV in whole kernels (Klich, 2007; McLean et al., 1994). Therefore, we recommend milling as a necessary step to decontaminate AF in solid foods such as peanuts.

Solar irradiation should be able to efficiently decontaminate not only liquid foods but also solid foods if they are broken down into small particles. In our case, an almost 40% reduction of total AF in the milled sample was observed after an hour of UV-A irradiation, implying that the solar irradiation can work even better with a higher irradiance in the UV-A range (Marzo et al., 2018). The data shows that the irradiance in Antofagasta, Chile on 2015 February 24 was greater than 0.08 $mW/cm^2 \cdot nm$ in the UV-A range, which was equal to a greater than 3.2 mW/cm^2 of irradiance located in the AF sensitive wavelength range, 345 to 385 nm. Therefore, using solar irradiation as the UV-A source is undoubtedly practical.

Conclusions

This study justified the use of radiation in the UV-A range to reduce AF in contaminated peanuts. Before being processed with UV radiation, peanuts should be peeled to increase the decontamination efficiency. Both UV-A and UV-C ranges showed the same level of AF reduction, but the oil quality was significantly deteriorated by UV-C treatment. UV-A could effectively decontaminate AF into less toxic products while preserving the oil quality of peanuts (Shen & Singh, 2021a). Additionally, breaking down peanuts into small particles can further enhance the AF reduction by UV-A irradiation. The blanching and milling process largely limit the application of UV-treated peanuts due to the loss of the integrity of kernels, but the milled peanuts products can be used as an ingredient for other purposes which do not require whole-seeds, e.g., confectionaries, drinks, or peanut butter.

The UV-A radiation source could be from LP UV-A lamp, MP lamp, or solar irradiation. However, LP lamps have a better energy efficiency than MP lamps. The abundant and feasible radiation sources facilitate the spread of this AF decontamination technology to places that lack

infrastructure. More studies should be done for optimizing the process parameters such as, in this case, peeling and milling.

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Tables

Table 5.1 Specifications of UV lamps used in this study.

Lamp type	Low-pressure lamp			Medium-pressure lamp
	UV-C	UV-B	UV-A	
Power	17W	17W	17W	2000W
Phosphors coating of lamp tube	Clear quartz tube, no coating	Narrow band UVB (Nichia Co., Tokyo, Japan)	NP802 (Nichia Co., Tokyo, Japan)	Clear quartz tube, no coating
Wavelength ranges	250 to 260 nm	295 to 320 nm	345 to 400 nm	185 to 1367 nm
Peak output wavelength	254 nm	310 nm	365 nm	Polychromatic, max. at 365 nm
Reference	Bolton & Linden, 2003	LightSources Inc. (Orange, CT)	LightSources Inc. (Orange, CT)	Zimmer & Slawson, 2002

Table 5.2 Aflatoxin concentration of artificially contaminated milled peeled-kernel and milled peanut-peel before and after a 2-hour irradiation (2.18 mW/cm^2) from 2 low-pressure UV-C lamps (n = 2).

Sample	Aflatoxin concentration (ng/g)					Total AF reduction*
	AFB_1*	AFB_2*	AFG_1*	AFG_2*	Total AF*†	
Milled peeled-kernel						
control	47.80 ± 2.93	10.94 ± 1.15	20.36 ± 2.09	4.95 ± 1.96	84.04 ± 1.92	
UV-C, 2.0 h	33.24 ± 1.52	8.25 ± 0.73	14.75 ± 1.30	3.98 ± 1.19	60.23 ± 4.75	28%
p-value	0.025	0.108	0.085	0.613	0.022	
Milled peanut-peel						
control	52.11 ± 2.07	10.94 ± 0.19	23.17 ± 1.88	4.67 ± 0.03	90.88 ± 0.36	
UV-C, 2.0 h	41.87 ± 4.96	10.55 ± 0.07	20.49 ± 1.20	4.66 ± 0.12	77.57 ± 5.97	15%
p-value	0.115	0.116	0.232	0.958	0.088	

* AFB_1: aflatoxin B_1, AFB_2: aflatoxin B_2, AFG_1: aflatoxin G_1, AFG_2: aflatoxin G_2, Total AF: total aflatoxins.
† The sum concentration of AFB_1, AFB_2, AFG_1, and AFG_2.

Table 5.3 Aflatoxin concentration in peeled kernels before and after being treated with four types of UV lamps (n = 2).

Treatment	Aflatoxin concentration (ng/g)					Total AF reduction*
	AFB_1*	AFB_2*	AFG_1*	AFG_2*	Total AF*†	
Control	111.23 ± 1.73^{a}	25.77 ± 1.60^{a}	57.75 ± 1.62^{a}	10.83 ± 0.99^{a}	205.58 ± 0.49^{a}	
LP UV-A§						
0.5 h	83.81 ± 1.12^{de}	19.77 ± 0.86^{c}	43.30 ± 3.20^{cd}	9.17 ± 0.12^{a}	156.04 ± 2.81^{d}	24%
1.0 h	80.13 ± 1.79^{e}	19.87 ± 0.85^{bc}	42.71 ± 1.19^{d}	9.22 ± 0.97^{a}	151.92 ± 4.79^{d}	26%
LP UV-B§						
0.5 h	104.11 ± 5.87^{ab}	23.23 ± 2.14^{abc}	51.80 ± 3.38^{abc}	10.36 ± 1.81^{a}	189.50 ± 13.19^{ab}	8%
1.0 h	94.79 ± 1.66^{bcd}	21.54 ± 0.68^{abc}	47.07 ± 3.01^{bcd}	9.24 ± 0.54^{a}	172.64 ± 5.89^{bcd}	16%
LP UV-C§						
0.5 h	103.50 ± 5.41^{ab}	24.46 ± 0.25^{ab}	53.04 ± 1.50^{ab}	10.68 ± 0.94^{a}	191.69 ± 6.22^{ab}	7%
1.0 h	87.33 ± 1.18^{de}	21.50 ± 0.72^{abc}	44.61 ± 2.34^{bcd}	9.79 ± 0.59^{a}	163.23 ± 3.65^{cd}	21%
MP§						
5 min	99.44 ± 1.73^{abc}	23.85 ± 0.87^{abc}	52.78 ± 0.91^{ab}	10.46 ± 1.03^{a}	186.54 ± 4.53^{abc}	9%
10 min	89.90 ± 1.24^{cde}	22.94 ± 1.40^{abc}	49.40 ± 1.93^{abcd}	11.23 ± 0.93^{a}	173.47 ± 5.49^{bcd}	16%
p-value	<0.0001	0.007	0.001	0.366	0.0001	

* AFB_1: aflatoxin B_1, AFB_2: aflatoxin B_2, AFG_1: aflatoxin G_1, AFG_2: aflatoxin G_2, Total AF: total aflatoxins.

† The sum concentration of AFB_1, AFB_2, AFG_1, and AFG_2.

§ LP: low-pressure lamp, MP: medium-pressure lamp.

$^{a, b, c, d, e}$ letters represent the significant difference ($p < 0.05$) within the same column.

Table 5.4 Oxidation induction times of milled peeled kernel and cold-pressed oil from the peeled kernel before and after being treated by low-pressure UV-A and low-pressure UV-C lamps (n = 2).

Treatment	Oxidation Induction Time (min)
Milled peeled-kernel	
Control	63.1 ± 1.2[a]
UV-A, 1.0 h	64.0 ± 0.1[a]
UV-C, 1.0 h	31.7 ± 0.4[b]
Cold-pressed peeled-kernel oil	
Control	60.8 ± 0.6[a]
UV-A, 15 min	59.4 ± 0.3[a]
UV-C, 15 min	11.6 ± 0.2[b]

[a, b] letters represent the significant difference ($p < 0.05$) within the same type of sample.

Table 5.5 Aflatoxin concentration change of inoculated whole peeled kernel and milled peeled kernel before and after being treated by low-pressure UV-A lamps (n = 3).

Treatment	Aflatoxin concentration (ng/g)					Total AF reduction*
	AFB_1*	AFB_2*	AFG_1*	AFG_2*	Total AF*†	
Whole peeled kernel						
Control	1576.71 ± 469.76	43.63 ± 16.00	1918.48 ± 706.92	39.07 ± 19.95	3577.89 ± 1174.33	
UV-A, 1.0 h	1362.9 ± 583.25	39.48 ± 14.32	1493.98 ± 566.80	31.93 ± 10.38	2928.29 ± 1164.21	18%
UV-A, 4.0 h	1117.38 ± 320.37	35.78 ± 10.33	1369.47 ± 349.27	36.07 ± 5.59	2558.69 ± 669.85	13%
p-value	0.526	0.790	0.496	0.812	0.511	
Milled peeled kernel						
Control	1384.35 ± 105.48	39.78 ± 3.51	1394.33 ± 97.76	24.96 ± 1.99	2843.42 ± 206.54	
UV-A, 1.0 h	772.05 ± 354.52	30.79 ± 14.62	892.90 ± 358.03	25.17 ± 8.85	1720.90 ± 734.79	39%
p-value	0.046	0.358	0.079	0.971	0.064	

* AFB_1: aflatoxin B_1, AFB_2: aflatoxin B_2, AFG_1: aflatoxin G_1, AFG_2: aflatoxin G_2, Total AF: total aflatoxins.

† The sum concentration of AFB_1, AFB_2, AFG_1, and AFG_2

Figures

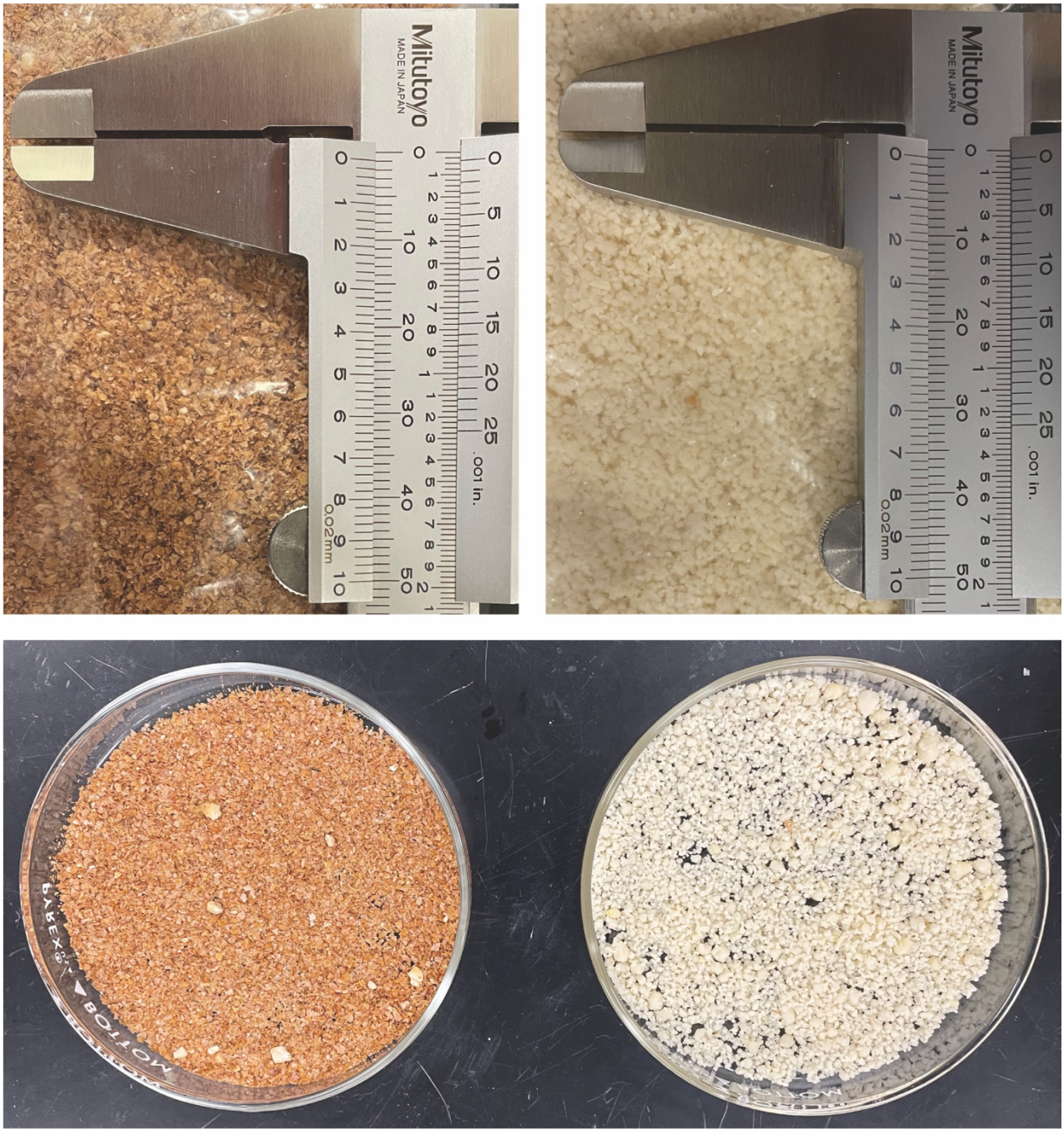

Figure 5.1 Milled peanut peel (upper left) and peeled kernels (upper right) loaded in Petri dishes (bottom).

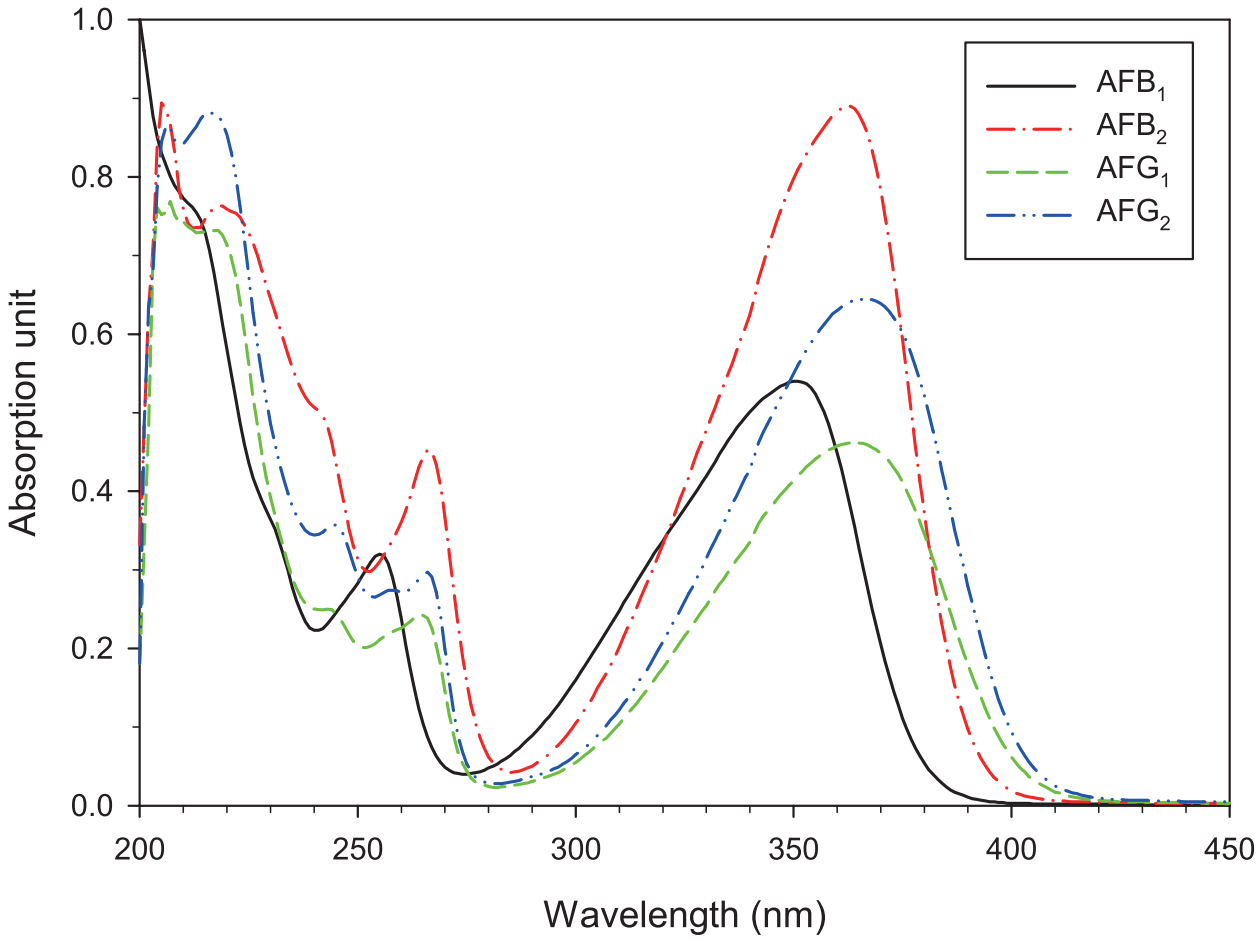

Figure 5.2 Absorption curves of aflatoxin stock solutions (AFB_1: 10.41 µg/mL, AFB_2: 13.06 µg/mL, AFG_1: 8.58 µg/mL, AFG_2: 11.09 µg/mL).

CHAPTER 6

DETOXIFYING AFLATOXIN CONTAMINATED PEANUTS BY HIGH CONCENTRATION OF H_2O_2 AT MODERATE TEMPERATURE AND CATALASE INACTIVATION[1]

[1] Shen, M. H., & Singh, R. K. 2021. Submitted to *Food Control*, 12/28/2021.

Abstract

H_2O_2 treatment fulfills the requirement of environmentally friendly and safety concerns since it can be easily removed or decomposed into water and oxygen. Even though the high efficiency in detoxifying aflatoxins (AF) in foods by H_2O_2 was reported by some studies, the information to utilize this reagent for practical application is very limited. This study aimed at investigating the effect of 30 g/hg H_2O_2 at 50˚C on catalase-inactivated peanuts. Raising treatment temperature from room temperature (20˚C) to 50˚C promoted the AF reduction from 30% to 73%. Catalase-inactivated peanuts prepared by roasting at 140˚C for 10 min slightly increased the AF reduction from 86% to 90% after 8 h of 50˚C, 30 g/hg H_2O_2 treatment. A 95% of AF reduction was observed in the fungi-inoculated peanuts after the same treatment. The oil quality was not seriously affected by the treatment. The AF reduction found in peanuts was higher than in the model solution, indicating that peanuts enhanced the AF degradation. The weight loss and oil quality change of the treated peanuts were negligible. Peanuts were able to keep intact after the treatment because the temperature of the treatment was lower than that of the starch gelatinization. Most H_2O_2 was removed by drying H_2O_2 treated peanuts at 35˚C for 12 h. These findings justify the use of H_2O_2 in AF detoxification.

Introduction

The increasing aflatoxin (AF) contamination due to climate change causes threats to our food supply chain (Wu & Mitchell, 2016). The widespread contamination of staple foods of this carcinogenic and genotoxic toxin considerably jeopardized not only the health of humans but also livestock and poultry (Joint FAO/WHO, 2017). Based on the principle of sustainability to reduce food waste, strategies to mitigate the issue were proposed, involving the techniques from crop cultivation to post-harvest processing (Udomkun et al., 2017). Among these strategies, chemical methods fulfill requirements such as being economically practical and easy to handle. Therefore, these treatments are of more interest, especially for those areas with a prevalence of AF contamination but a lack of infrastructure to combat the problem.

Hydrogen peroxide is a strong oxidizer and has proven to be able to efficiently detoxify AF while having only a few impacts on the qualities of treated foods. Sreenivasamurthy et al. (1967) reported that the growth of duckling was not affected after being fed with pure AF treated with 6 g/hg H_2O_2 at 80˚C under pH 9.5 for half-hour. Additionally, the treatment was able to remove the AF from the contaminated peanut meals without significant influence on the protein efficiency ratio. H_2O_2 has been approved as an ingredient or sanitizer for use in food processing and can be easily removed by conventional unit operations such as drying. This largely eliminates safety concerns that which usually accompany the use of the chemicals in detoxifying AF contaminated foods. H_2O_2 is also considered an environmentally friendly compound since it decomposes into oxygen and water when being exposed to light. However, H_2O_2 is stable enough under room temperature and takes a considerable time to degrade AF (Tabata et al., 1994). To improve the AF detoxication efficiency, catalysts such as high temperature, alkaline, or radiation are needed to accelerate the AF degradation rate (Shen & Singh, 2021a). Nonetheless, a high-temperature

treatment usually impairs the nutrients and quality of foods, limiting the use of those foods for a variety of purposes. Alkaline treatments would help to decompose AF but may cause off-odor and taste (Elias-Orozco et al., 2002). The reverse of degraded AF compounds after alkaline treatment to their original toxic form under acidic conditions such as in mammal's digestion systems is also a concern (Tabata et al., 1994). Radiation is effective in promoting AF degradation, but the cost for the facilities is not affordable to most growers or manufacturers (Patel et al., 1989). Aside from the low reactivity of H_2O_2, the food matrix also plays an important role in AF degradation. The high reduction of AF by H_2O_2 treatment was observed in the model solution containing only pure AF but not in corn grit (Tabata et al., 1994). This could be due to the prevalence of reactive oxygen species scavengers such as catalase to protect bio-organisms from being attacked by H_2O_2 (Imlay, 2003).

Even though it is evident that H_2O_2 has the potential to decompose AF while retaining the quality of foods, the information about manipulating the use of H_2O_2 in a detoxification process is limited. In this study, we investigated the effect of moderately increasing the reaction temperature as well as inactivating the catalase in peanuts on AF reduction in artificially toxin-spiked peanuts. The treatment was validated using fungus-contaminated peanuts with a high level of AF. The oil quality, color change, and residual H_2O_2 in peanuts after the treatment were also evaluated.

Material and Methods

Chemicals and reagents

Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), NaCl, $Na_2S_2O_3$, KI, $(NH_4)_2MoO_4$, and catalase from bovine liver (C30) were ACS grade and purchased from Sigma-Aldrich (MO, USA). Toluene, ethyl acetate, and acetonitrile were HPLC grade and purchased from Sigma-Aldrich. Hydrogen peroxide solution (30 g/hg, ACS grade) was

purchased from Acros Organics (NJ, USA). Methanol (HPLC grade), formic acid (LC-MS grade), H_2SO_4 (ACS grade), potato starch for iodometry, and Tween 80 were purchased from Fisher Chemical (NJ, USA). $MgSO_4$ was ACS grade and purchased from J. T. Baker Chemical (NJ, USA). Potato dextrose agar (PDA) was purchased from Millipore (Darmstadt, DE).

Evaluation of the effect of temperature on AF decomposition using a model solution

AFB1, AFB2, AFG2, and AFG2 stock solution in methanol containing about 10 µg/mL of AF were prepared by the AOAC method 971.22 (AOAC, 2000). Mixed AF stock solution (96 µL) containing AFB1/AFB2/AFG1/AFG2, 5.102/1.276/2.551/1.276 µg/mL was mixed with 5 mL 30 g/hg of H_2O_2 solution loaded in a glass test tube held in a water bath to keep the temperature at 20˚C or 50˚C for 4 or 8 h. After the reaction, 1 mL of the solution was mixed with 9 mL of DI water then passed through an immunoaffinity column (AflaTest® P column, Vicam, MA, USA) followed by the aflatoxin assay analysis. The control group was prepared by passing the sample solution into the immunoaffinity column described above immediately after mixing the AF stock solution with the H_2O_2 solution. The experiment was done in duplicate.

Inactivation of catalase in peanuts by roasting

Fresh peanuts containing less than 5 ng/g of AF were obtained from Golden Peanut Corporation (Alpharetta, GA, USA) and stored in vacuumed seal bags at 4˚C until being used. Fresh peanuts were roasted in a convection oven (Model MC32ACG-CHSS, Toshiba, Tokyo, JP) at 140 ± 5˚C for 2, 4, 6, 8, 10, 15, and 20 min. Roasted peanuts were sectioned at the middle of each peanut into about 3 mm slices. H_2O_2 solution (30 g/hg, 100 µL) was spread on each slice. After 10 min of reaction, the production of bubble on each slice was recorded. The experiment was performed in duplicate.

Determination of the effect of inactivating catalase on AF decomposition in artificially spiked peanuts

Spiked fresh peanuts containing about AFB1/AFB2/AFG1/AFG2, 100/25/50/25 μg/g of AF were prepared using the method described in our previous work (Shen & Singh, 2021b). Roasted peanuts were prepared by roasting fresh peanuts in a convection oven (Model MC32ACG-CHSS, Toshiba, Tokyo, JP) at 140 ± 5˚C for 10 min followed by the same AF spiking procedure. About 25 g of peanuts sample was loaded in a 250 mL Erlenmeyer flask containing 50 mL of 30 g/hg H_2O_2 solution. The flask was sealed with 2 layers of parafilm (Bemis Company Inc., WI, USA) and then covered with a layer of aluminum foil. Sealed flasks were immersed in a 50˚C of water bath for 4 or 8 h. Peanuts were drained and subjected to the AF assay after treatments. The experiment was performed in duplicate.

Validation of the H_2O_2 treatment using fungi inoculated peanuts

Aspergillus nomius (NRRL 6108) obtained from Agricultural Research Service Culture Collection (IL, USA) was used to inoculate peanuts. About 1000 g of fresh peanuts were rinsed with 500 mL of DI water and drained. Rinsed peanuts were then mixed with 40 mL of spore solution containing about 10^6 to 10^7 spores/mL in a food storage box (28 cm × 18 cm × 8 cm, Wal-Mart Stores Inc., AR, USA). The box was sealed with surgery masks and mildly shaken until all the liquid was absorbed. The box was then incubated at 30˚C, RH > 90% for 6 days. During the incubation, boxes were shaken once per day to evenly distribute the fungus mycelia. The spore solution was harvested by washing PDA plates inoculated with NRRL 6108 for 4 days with 1 g/hg of Tween 80 solution. Inoculated peanuts were roasted, subjected to H_2O_2 treatment for 8 or 24 h, and analyzed as described in Section "Determination of the effect of inactivating catalase on AF decomposition in artificially spiked peanuts." The experiment was performed in triplicate.

Aflatoxin assay by immunoaffinity column (IAC) and HPLC-Fluorescence detector (HPLC-FLD)

Aflatoxin determination was performed by the modified AOAC method 991.31 (AOAC, 2002) combined with normal phase HPLC-FLD (Leitao et al., 1988; Manabe et al., 1978). Peanut sample containing AF was blended (Model WF2211214, Waring corporation, CT, USA) with 5 g NaCl and 125 mL 70 mL/dL of methanol at high speed for 2 min. A 24-cm fluted filter paper (Fisher Scientific, NH, USA) was used to filter the slurry. The filtrate (12 mL) was diluted with DI water (20 mL), well mixed, and further filtered with a 1.5 μm glass microfiber filter paper (Whatman® 934-AH, GE Healthcare, IL, USA). An AflaTest® P immunoaffinity column (Vicam, MA, USA) was used to extract AF from the secondary filtrate (10 mL for spiked peanut sample, 2 mL for inoculated peanut sample). The filtrate passing rate was adjusted to about 1 drop per min by an air pump (Vicam, MA, USA). After passing all filtrate, the column was washed with 20 mL DI water at the same passing rate. Acetonitrile (0.45 mL) was added to the washed column to elute AF into a test tube. The eluate was then mixed with 0.20 g $MgSO_4$ and 0.30 mL toluene in the test tube immersing in an ice bath. The mixture was well vortexed until the solution became clear. The solution without precipitate was pipetted into the 0.4 mL insert within a 2 mL HPLC sample vial.

The mobile phase of HPLC was prepared by mixing solution A (toluene/ethyl acetate/formic acid, 45 mL/3 mL/2 mL) and solution B (toluene/ethyl acetate/methanol, 45 mL/3 mL/2 mL) in the quaternary pump of an HPLC (Model Infinity 1260, Agilent, CA, USA) at a ratio of 1 mL:1 mL and a flushing rate of 1.5 mL per min. Solution A and B should be mixed only in the HPLC pump and prepared independently to prevent spontaneous reactions. The unmixed solutions can be stored at room temperature in the dark for up to 2 weeks. A 40 μL of the eluate was injected into the LC and separated by an LC-Si column (5 μm, 25 cm × 4.6 mm, Supelco, Bellefonte, PA, USA) held at 30˚C. Each sample was flushed with the mobile phase for 20 min.

The LC column was flushed with toluene/acetyl acetate (9 mL:1 mL) at a rate of 1.0 mL for 1 h before and after the analysis every day. The exciting wavelength of FLD was set at 365 nm and the emission wavelength was 425 nm. The sampling rate was 2.31 Hz. The AF concentration in the sample can be obtained by the following equations:

$$W = S\ g \times (12\ mL/125\ mL) \times (I\ mL/32\ mL) \quad (1)$$

$$\text{Aflatoxin in peanut (ng/g)} = (A \times E) / W \quad (2)$$

where W = equivalent weight of the sample in the eluate (g), S = dry weight of the peanut sample (g), which was obtained by subtracting the increased weight by H_2O_2 solution from the weight of the sample taken after the H_2O_2 treatment, I = volume of the secondary filtrate passed through the immunoaffinity column (mL), E = volume of acetonitrile and toluene to elute AF and dilute the eluate (mL), which was 0.75 mL, and A = AF concentration in the eluate (ng/mL).

Evaluation of oil quality change by differential scanning calorimetry (DSC)

Oil quality evaluation was performed by comparing the oxidation induction time (OIT) before and after H_2O_2 treatment according to the modified quality determination method (Zhang et al., 2021). Roasted peanuts were cold-pressed (Model 3853, Carver Inc., IN, US) to obtain the oil. For H_2O_2 treated sample, peanuts were drained and then dried in the convection oven (Model MC32ACG-CHSS, Toshiba, Tokyo, JP) at 35 ± 5˚C for 12 h before being cold-pressed. Obtained oil (20.0 ± 0.5 mg) was pipetted into an aluminum crucible pan covering without a lid (40μL, Model ME-27331, Mettler Toledo, OH, USA). The pan was then loaded in a DSC (Model DSC-1 700, Mettler Toledo, OH, USA). The DSC was initially purged with N_2 gas at a flow rate of 50 mL/min and held at 25˚C. The temperature was then increased from 25˚C to 140˚C at a rate of 40˚C/min and held for 3 min. After 3 minutes, the purging gas was switched to O_2 gas at a flow rate of 50 mL/min for triggering oil oxidation. OIT was obtained by the following equation:

$$OIT\ (min) = T_i - (T_t + T_s) \quad (3)$$

where T_i = the time of interception point (x-axis) of the extrapolated baseline at the early stage and the tangent line of the leading edge of the exothermic peak, T_t = time for increasing temperature of sample from 25˚C to 140˚C, T_s = times for stabilizing sample, which is 3 min.

Hydrogen peroxide assay

Residual hydrogen peroxide in treated peanuts was determined using the modified iodometry method (Walsh et al., 2018). About 10 g of peanut sample was mixed with 100 g of DI water in a beaker and mildly shaken for 1 min. The rinsed water (10 g) was pipetted to an Erlenmeyer's flask followed by adding 1 mL of 4.5 M sulfuric acid, 1 mL of 1.5 g/dL ammonium molybdate, and 3 mL of 10 g/dL potassium iodide (freshly prepared every day). During the addition of reagents, the flask was kept swirling. The mixture was then placed in the dark to react for 10 min and titrated with 0.05 M $Na_2S_2O_3$ until the dark blue color disappeared. Starch indicator (1 mL, 1 g/dL) was added before the titration as an indicator. The control group was prepared by adding 100 μL catalase solution (10 mg/mL) into the 10 g sample-rinsed water and frequently swirling for 10 min followed by the same procedure described above. The H_2O_2 concertation was obtained by subtracting the volume of $Na_2S_2O_3$ used in the control group from that used in the sample and then calculated by the following equations:

$$H_2O_2 \text{ concentration (g/g)} = (V \times 1/1000 \times N/2 \times M_{H2O2}) / (W \times 10g/100g) \quad (4)$$

where V = volume of the $Na_2S_2O_3$ used in control group after subtracting that used in control (mL), N = normality of $Na_2S_2O_3$ solution used for titration (mol/L), which is 0.05 M, M_{H2O2} = molar mass of H_2O_2 (g/mol), W = peanut's dry weight (g).

Color measurement

Color measurement was done by taking the average value from 3 positions in the same batch of peanuts with a colorimeter (Model MiniScan EZ 4500L, HunterLab, VA, USA). Measurement was recorded in CIELAB color space (L*, a*, b*) format.

Statistical analysis

Results were analyzed using ANOVA table, student t-test, and Tukey's pair test on JMP Pro software (version 15.0.0, SAS Institute Inc., NC, USA) with a significant level, $\alpha = 0.05$.

Results and Discussion

Effect of temperature on AF decomposition

The results shown in Table 6.1 demonstrate that enhancing temperature is significant to promote AF degradation. It is noticeable that, even though we used 30 g/hg H_2O_2, which is the highest concentration mostly applied in the food industry due to the safety consideration, the total AF reduction, about 30%, was still low under the room temperature after the 8 h treatment. The result does not coincide with the result reported by Tabata et al. (1994), which treated pure AF in 1 g/hg H_2O_2 solution for 16 h under room temperature and observed more than 90% reductions in four types of AF. The AF assay they employed, thin layer chromatography, could wrongly estimate the concentration of AF. On the other hand, we found that increasing the temperature from 20˚C to 50˚C significantly increased the total AF reduction from 30% to 73%. The temperature was applied because under 50˚C, most starch granules have gelatinization temperatures between 52 to 87˚C (Belitz et al., 2009). Treating peanuts under that temperature range should be able to keep the nutrients and the integrity of whole peanuts.

It seems that four types of AF follow the different degradation pathways. We hypothesize that the double bonds between 8 and 9th carbons connected to oxygens in AFB1 and AFG1 are

more suspectable to the attack of hydroxyl radical generated from H_2O_2 (Y. Z. Wu et al., 2015). On the other hand, the hydrolysis of the additional lactone ring in AFG1 and AFG2 under acidic conditions, compared to AFB1 and AFB2, may facilitate the decomposition (Nicolás-Vázquez et al., 2010). The pH of 30 g/hg of H_2O_2 solution we used was about 4.3. Understanding the mechanism behind the degradation of four types of AF may largely help us to develop a new detoxification strategy.

In the LC analysis, no aflatoxin B2a (AFB2a) was found in the chromatograph, indicating that AF was degraded by H_2O_2 or its free radicals rather than the acidic environment of the solution. Using low pH to oxidize AFB1 into AFB2a has been well studied (Rushing & Selim, 2016). AFB2a is a compound with much less toxicity and chemical stability than AFB1 (Shen & Singh, 2021a). That is, combining low pH created by dietary acids as well as H_2O_2 could be another direction to detoxify AF.

Effect of inactivating catalase on AF decomposition in artificially spiked peanuts

The roasting time for inactivating catalase was determined by observing the oxygen bubbles produced on the peanut sections. As can be seen in Fig. 6.1, the white foam forming on the peanut section demonstrated the enzyme activity of catalase. After 8 min of roasting, almost no foam can be observed. We decided to roast peanuts for 10 min, considering the variation among different peanut individuals. When immersing peanut into H_2O_2 solution, however, bubbles were still generated from inside of peanuts after half-hour of immersing, indicating that the catalase inactivation was incomplete.

The roasted peanuts showed a higher AF reduction, even though the difference is small (Table 6.2). The roasted peanuts did not produce as many bubbles as fresh peanuts did. The small difference between the two treatments could result from the incomplete inactivation of catalase

inside the peanuts where the lethality did not reach the desired level during the roasting. The other explanation is that the concentration of H_2O_2 used in this experiment was always at a high level and sufficient to effectively degrade AF, although some of H_2O_2 has been consumed by either the matrix or the enzyme. In this scenario, the enzyme activity has only a minor effect on AF reduction. That is, using lower concertation of H_2O_2 to detoxify roasted peanuts is possible.

The AF reduction in peanuts is non-intuitive, compared to the results in the model solution. H_2O_2 degraded more AF in peanuts than in solution, which conflicts with previous work reported by Tabata et al. (1994). They reported a much lower AF reduction in corn grit than in model solution and provided an explanation that the AF was protected by the food matrix such as starch or reducing saccharides by reacting with H_2O_2. This hypothesis obviously cannot interpret the situation we met. Instead, we thought that the interaction between the excess reagent, H_2O_2, and non-inactivated peroxidase in peanuts may account for this phenomenon. Using peroxidase to detoxify AF was reported (Das & Mishra, 2000), so in this experiment, the excess H_2O_2 could react with peroxidase and cause more AF reduction. The low concentration of H_2O_2, 1 g/hg, used by Tabata et al. (1994) could be quickly consumed by enzymes or the matrix at the beginning of the experiment and hence was not able to react with peroxidase and AF.

We envision that the effect of enzyme inactivation by roasting on AF reduction will be more significant in fungi-contaminated peanuts. Fungi activity produces more catalases. The characteristic was applied to separate fungi-contaminated peanuts from those unaffected by H_2O_2 solution (Clavero et al., 1993). Additionally, inactivating enzymes can facilitate H_2O_2 to enter the peanut tissue by eliminating the obstacle of forming bubbles, reacting with non-inactivated peroxidase located inside of the tissues.

Validation of H_2O_2 treatment by fungi inoculated peanuts

As shown in Table 6.3, an uneven distribution of AF in inoculated peanuts was observed even we had made an effort to uniformly distribute the mycelia on every peanut kernel. It seems that obtaining a batch of inoculated kernels with even AF distribution is difficult. A similar result was reported in another study (Martins et al., 2017). In addition, even subjected the 140˚C, 10 min of roasting, the peanuts still had a high level of AF, implying that roasting is an inefficient AF detoxification method, especially for those heavily contaminated peanuts.

A more AF reduction observed in fungus-contaminated peanuts than in the spiked sample supports our hypothesis described in Section "Effect of inactivating catalase on AF decomposition in artificially spiked peanuts." The fungi produced a higher concentration of peroxidase and therefore resulted in more AF reduction with the excess supply of H_2O_2. In this experiment, we prolong the H_2O_2 treatment to 24 h. However, the total AF reduction increased only about 2%, from 95% to 97%. The result suggests that prolonging the treatment time in the detoxification process is not significant. The residual H_2O_2 in peanuts after the treatment shown in Table 6.4 indicates that there was still about 1.3 g/hg of H_2O_2 remaining in peanuts, revealing that the low AF reduction rate after 8 h was unlikely caused by the exhaustion of H_2O_2. Evidence shows that AF, as a type of phytotoxin, can migrate into deeper areas of plant tissue and therefore is difficult to completely remove (Cucullu et al., 1966; McLean et al., 1994). To effectively detoxify these toxins, a sufficient amount of H_2O_2 has to arrive in the contaminated area by avoiding being decomposed by catalase during traveling across peanut tissue.

The considerable AF reduction in heavily contaminated peanuts reported in this study reveals the potential of H_2O_2 in practical use. The residual AF in treated peanuts exceeded the standard of being consumed as human food but met the standard for animal feeds, according to the US FDA guidelines (US FDA, 2019). With further improvements, the proposed detoxification

treatment may be able to meet the standard for human foods, such as grounding contaminated peanuts into small particles to increase the exposure of the contamination to the H_2O_2 solution.

Evaluation of quality change and residual H_2O_2 in peanuts after treatment

A small amount of H_2O_2 was detected in the dried peanut sample (Table 6.4). However, we expect that the residual H_2O_2 can be easily removed by prolonging the drying process. The weight loss of peanuts after the treatment was negligible, indicating that no notable mass transfer occurred during the treatment. That is, macronutrients such as lipid, protein, and starch were mostly preserved. Evidence revealed that H_2O_2 treatment could increase proteolysis by several enzymes such as trypsin (Ailes et al., 2019; Fligiel et al., 1984). This could be the reason that a slightly higher protein efficiency ratio was observed when feeding ducks with H_2O_2 treated peanut meal (Sreenivasamurthy et al., 1967). The OIT indicates that the oil quality was affected by the 24-h H_2O_2 treatment to some degree, but the change, from 51.6 to 41.8 min, was not significant and can be considered as an initial stage of quality loss instead of an occurrence of serious deterioration. We had expected more severe oil rancidity since peanut oil is rich in unsaturated fatty acids and can be easily oxidized with the presence of peroxides. Fig. 6.2 shows the peanuts after the 24-h treatment and the subsequent drying.

The appearance of the treated peanuts was largely changed. The peanuts remained intact but were slightly swollen and almost all skins were ruptured. The swell was caused by the absorption of H_2O_2 solution. After the drying process, the peanuts shrank to the original size before the treatment. The large color change was due to the loss of skin of kernels. The long time soaking in warm H_2O_2 solution loosened and decomposed the peanut skin, causing the most of skin was removed after the treatment. The process is similar to the process to remove the skin in the industry

(Angelo et al., 1977). The roasting for catalase inactivation can also be replaced with or as the part of blanching step in current peanut processing to remove the skin.

H_2O_2 is a bleach and can further discolor the peanuts. A similar effect was also observed when treating black pepper with H_2O_2 (Jalili et al., 2011). Even though the previous study used duckling to evaluate the protein efficiency after treatment, future works should focus on the effect of H_2O_2 on amino acids. A sensory test during prolonged storage period may be required.

Conclusions

This study demonstrated that H_2O_2 treatment fulfills the requirements of effectively detoxifying AF with acceptable quality change. The treatment, immersing peanuts in 30 g/hg H_2O_2 at 50˚C for 24 h, successfully reduced the AF from about 2.8 μg/g to less than 100 ng/g in severely contaminated peanuts with negligible oil quality change, slight mass transfer, but obvious appearance change. The main change in appearance was the loss of skin and that should not be an issue. The interaction between high concentration of H_2O_2 and peanuts is much more complicated than our expectation. To investigate these unexpected results may help us to further improve the proposed detoxification method, for example, a lower concentration of H_2O_2 use and a shorter processing time. The unknown factor that enhanced AF reduction in peanuts should be clarified, even though it was well evidenced that peroxidase was the most possible factor contributing to the additional reduction. The effect of enzyme inactivation should be further confirmed. The enzyme in peanuts may assist in degrading AF but obstruct the H_2O_2 to reach contaminated areas inside the tissue. Additionally, the optimum concentration of H_2O_2 used in the treatment needs to be further investigated. Our result indicates that lowering the H_2O_2 concentration is possible since there was still about 1.3 g/hg H_2O_2 remaining in the peanuts after 24 h, but the reduction was not

reduced as expected. The future direction will also focus on evaluating the nutrient availability to humans or animals.

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Table

Table 6.1 Aflatoxin concentration in model solution treated with 30 g/hg of H_2O_2 at 20˚C and 50˚C for 4 and 8 h (n = 2).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1[*]	AFB2[*]	AFG1[*]	AFG2[*]	Total AF[*§]
Control	121.15 ± 3.16[a]	39.30 ± 2.27[a]	58.31 ± 5.54[a]	25.14 ± 2.50[a]	243.90 ± 13.47[a]
20˚C, 30 g/hg H_2O_2, 4 h	99.87 ± 2.46[a]	33.40 ± 1.80[a]	35.86 ± 0.85[b]	17.28 ± 0.88[b]	186.40 ± 5.99[b]
20˚C, 30 g/hg H_2O_2, 8 h	93.86 ± 16.07[a]	33.39 ± 4.06[a]	27.99 ± 1.86[b]	14.80 ± 1.12[b]	170.05 ± 23.10[b]
50˚C, 30 g/hg H_2O_2, 4 h	50.12 ± 2.33[b]	31.62 ± 3.42[a]	2.97 ± 1.56[c]	4.72 ± 1.08[c]	89.42 ± 1.55[c]
50˚C, 30 g/hg H_2O_2, 8 h	33.39 ± 6.73[b]	29.50 ± 2.36[a]	0.52 ± 0.74[c]	1.94 ± 1.31[c]	65.35 ± 7.05[c]
p-value	<0.001	0.120	<0.001	<0.001	<0.001

[*] AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

[§] The sum concentration of AFB1, AFB2, AFG1, and AFG2.

[a, b, c] letters represent the significant difference ($p < 0.05$) within the same column.

Table 6.2 Aflatoxin concentrations in artificially AF spiked peanuts (with and without roasting) treated with 30 g/hg H_2O_2 at 50˚C for 4 and 8 h (n = 2).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1[*]	AFB2[*]	AFG1[*]	AFG2[*]	Total AF[*§]
Roasted peanuts					
control	101.16 ± 2.56	32.21 ± 3.54	46.45 ± 5.29	10.46 ± 0.52	190.29 ± 5.75
50˚C, 30 g/hg H_2O_2, 4 h	16.60 ± 0.64	13.42 ± 1.87	0.81 ± 0.11	0.68 ± 0.19	31.51 ± 2.82
50˚C, 30 g/hg H_2O_2, 8 h	7.03 ± 1.18	11.39 ± 0.64	0.00 ± 0.00	0.56 ± 0.03	18.99 ± 0.50
Reduction (%)[†]	93 ± 1	64 ± 6	100 ± 0	95 ± 0	90 ± 0
Raw peanuts					
control	111.82 ± 3.06	35.32 ± 1.66	52.29 ± 2.10	10.53 ± 1.01	209.96 ± 7.82
50˚C, 30 g/hg H_2O_2, 4 h	21.51 ± 0.03	13.01 ± 1.16	0.93 ± 0.16	0.90 ± 0.14	36.36 ± 0.89
50˚C, 30 g/hg H_2O_2, 8 h	15.06 ± 3.64	13.22 ± 2.70	1.00 ± 0.11	0.34 ± 0.48	29.62 ± 6.92
Reduction (%)[†]	86 ± 4	62 ± 9	98 ± 0	97 ± 5	86 ± 4
p-value[†]	0.139	0.825	0.011	0.626	0.261

[*] AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

[§] The sum concentration of AFB1, AFB2, AFG1, and AFG2.

[†] Reductions of aflatoxin in peanuts after 8 h of H_2O_2 treatment and their corresponding *p*-values.

Table 6.3 Aflatoxin concentration changes of roasted inoculated peanuts treated with 30 g/hg H_2O_2 at 50˚C for 8 and 24 h (n = 3).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1[*]	AFB2[*]	AFG1[*]	AFG2[*]	Total AF[*§]
Control	1588.01 ± 829.87[a]	32.82 ± 22.85[a]	1122.20 ± 742.82[a]	14.84 ± 15.73[a]	2757.87 ± 620.14[a]
50˚C, 30 g/hg H_2O_2, 8 h	130.57 ± 85.83[b]	12.22 ± 12.08[a]	7.37 ± 6.38[b]	0.85 ± 0.74[a]	151.01 ± 104.38[b]
50˚C, 30 g/hg H_2O_2, 24 h	63.95 ± 95.74[b]	12.53 ± 11.16[a]	7.23 ± 12.52[b]	0.42 ± 0.72[a]	84.12 ± 117.49[b]
p-value	0.014	0.281	0.029	0.168	<0.001

[*] AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

[§] The sum concentration of AFB1, AFB2, AFG1, and AFG2.

[a, b] letters represent the significant difference ($p < 0.05$) within the same column.

Table 6.4 Peanuts' quality changes after being roasted at 140˚C for 10 min, treated by 30 g/hg H_2O_2 at 50˚C for 24 h, and dried at 35˚C for 12 h (n = 3).

Treatment	Residual H_2O_2 (mg/g)	Weight (g)	OIT[§] (min)	Color (CIELAB color space)		
				L*	a*	b*
Roasting	-	50.49 ± 0.57	51.6 ± 3.0	45.5 ± 0.77	15.67 ± 0.44	26.24 ± 1.51
50˚C, 30 g/hg H_2O_2, 24 h	12.97 ± 17.50	73.25 ± 0.07	-	-	-	-
Drying	0.04 ± 0.06	49.42 ± 0.48	41.8 ± 3.5	60.41 ± 2.01	6.59 ± 0.48	30.68 ± 3.27
p-value	-	-	0.042	0.010	0.003	0.224

[§] OIT: oxidation induction time.

Figures

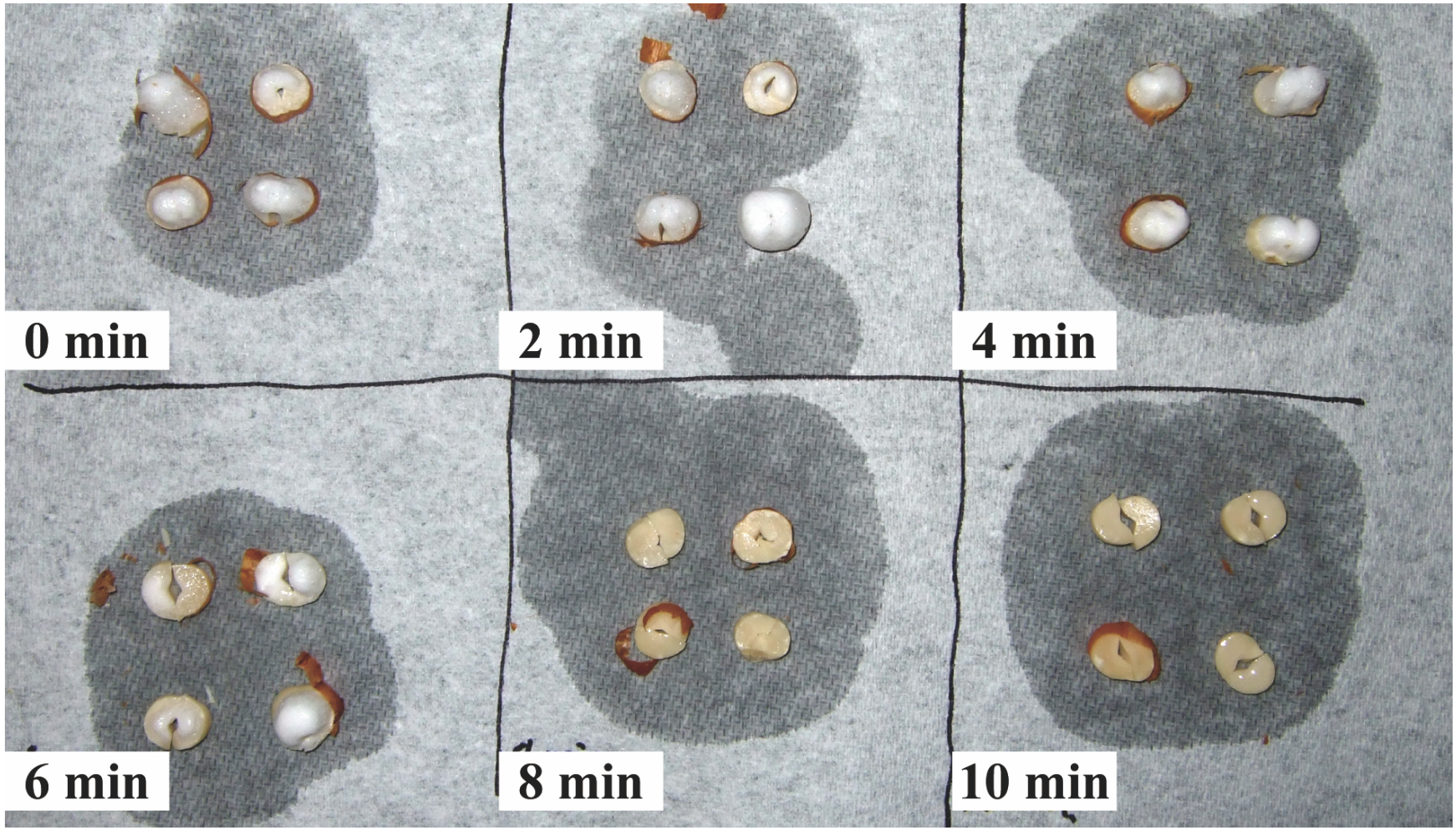

Figure 6.1 Sectioned peanuts (fresh and roasted at 140˚C for 2, 4, 6, 8, and 10 min) spread with 100 µL of 30 g/hg H_2O_2 and reacted for 10 min. White foam was oxygen bubbles produced from H_2O_2 decomposition by catalase.

Figure 6.2 Peanuts subjected to 30 g/hg H_2O_2 treatment at 50˚C for 24 h (left) and after being dried at 35˚C for 12 h (right).

CHAPTER 7

DECOMPOSING AFLATOXINS IN PEANUTS USING ADVANCED OXIDATION PROCESSES BY UV AND H_2O_2[1]

[1] Shen, M. H., & Singh, R. K. 2021. Submitted to *Food and Bioprocess Technology*, 01/21/2022.

Abstract

The advanced oxidation processes (AOP) employ free radicals generated from the photolysis of H_2O_2 by UV radiation to decompose the contamination in foods. This study aims at applying AOP to reduce the aflatoxins in peanuts. Radiations in UV-C or UV-A range combined with H_2O_2 solution efficiently degraded aflatoxins in the model solution. The degradation compound, aflatoxin B2a, was also degraded by AOP. Whole peanut kernels artificially spiked with aflatoxins subjected to 1 h AOP (2.76 mW/cm^2 UV-C, 1 g/hg H_2O_2) reduced 33% of aflatoxins. In fungus inoculated whole kernels treated with AOP, the reduction of aflatoxins was not significant, whereas in fungus inoculated milled kernels, AOP treatment significantly reduced 60% of aflatoxins. The residual H_2O_2 in peanuts was completely removed by drying the peanuts at 35˚C for 12 h to meet US FDA's requirement. The oil quality was slightly affected by the AOP treatment in whole kernels, but a more severe influence on oil quality was observed in the milled kernels. The color of whole kernels slightly darkened but did not considerably affect its appearance.

Introduction

Aflatoxin (AF) contamination in human foods and animal feeds has been recognized as a critical issue in our food supply chain. AF is a mycotoxin produced by the growth of fungi, mostly from the *Flavi* section in the *Aspergillus* genus (Shen & Singh, 2021a). The contamination causes severe adverse effects on human and animals health, e.g. hepatotoxicity, nephrotoxicity, and carcinogenicity (Mousavi Khaneghah et al., 2018). The outbreaks due to AF contamination are frequent and usually cause a considerable number of death or injuries to the public (Kumar et al., 2017). As a result, various amelioration strategies have been proposed to control AF in each step of a food supply chain from crop cultivation to post-harvest processing (Udomkun et al., 2017). Among those strategies, treatments dealing with the contamination in the post-harvest step are of more interest, especially when the preventive approaches fail to stop the growth of the fungi.

In this study, we propose a post-harvest AF decontamination method using the combination of UV and hydrogen peroxide to decompose AF in contaminated peanuts. The combination treatment, also known as advanced oxidation processes (AOP), has been widely used in water treatment for removing biological or chemical contaminations by generating free radicals such as hydroxyl radicals (Shen & Singh, 2021a). We expect that AOP should overcome the limitations of using H_2O_2 or UV radiation individually. H_2O_2 treatment usually takes a long time to degrade AF unless H_2O_2 molecules are activated by supplementary treatments such as high temperature or alkaline conditions (Elias-Orozco et al., 2002; Tabata et al., 1994). An additional cost for thermal processing or alkaline addition and its removal increases the difficulty of employing H_2O_2 treatment in practical use. In combination treatment, UV can serve as an activating factor to accelerate the cleavage of H_2O_2 into free radicals, promoting the degradation of AF with less energy consumption and equipment requirement (Glaze et al., 1987). On the other hand, even

though UV can degrade AF, the ability is limited by its low penetrability, especially in solid foods or liquids with high turbidity (Shen & Singh, 2021b). The food matrix can easily shade or obstruct the propagation of the radiation. Additionally, it was observed that even with uniformly delivered UV radiation to the samples, AF reduction was still unacceptable, indicating that AF was distributed at the locations where UV radiation was difficult to reach (Shen & Singh, 2021b). The free radicals generated from AOP can easily diffuse into the interior of food, thus further decomposing the AF (Hadjok et al., 2008).

Employing AOP possesses other advantages, compared to other post-harvest AF detoxification strategies. UV radiation has no residue issue and is recognized as a non-thermal process. The treatment should retain most qualities of treated foods. US FDA has approved the use of H_2O_2 in aiding processing for certain purposes as long as it is removed by appropriate physical and chemical means during the processing (US FDA, 2020). That is, foods after being subjected to AOP can be recognized as safe. More importantly, there are no harmful reagents or energy-consuming processes used in AOP, so the treatment can be considered as an environment-friendly treatment. The only limitation could be the oil deterioration induced by free radicals generated when applying this technology in oily foods such as peanuts. The micronutrients or vitamins might be of less interest since these micronutrients are not the main purpose of consuming peanuts.

The use of AOP for foods and water decontamination has been reported, but the information is mostly focused on the influence on microorganisms (Hadjok et al., 2008; Zeng et al., 2020). Studies using AOP to decontaminate AF in foods, especially for peanuts, which are vulnerable to being infected by AF producing fungi due to the underground maturation, might be out of date (Altuğ et al., 1990; Yousef & Marth, 1986). In the present study, we assessed the parameters for AOP, including the effective wavelength range and concentration of H_2O_2, and the

AF reduction contributed by AOP in artificially AF spiked peanuts as well as *Aspergillus nomius* inoculated peanuts. The oil quality, color changes, and residual H_2O_2 in peanuts after treatment and hot air drying were also evaluated.

Material and methods

Chemicals and reagents

Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2), NaCl, sodium thiosulfate, potassium iodide, and ammonium molybdate were ACS grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Toluene, ethyl acetate, and acetonitrile were HPLC grade and purchased from Sigma-Aldrich. Hydrogen peroxide (30 g/hg) was ACS grade and purchased from Acros Organics (NJ, USA). Catalase from bovine liver (C30) was purchased from Sigma-Aldrich. Methanol (HPLC grade), formic acid (LC/MS grade), sulfuric acid (ACS grade), starch (for iodometry), and Tween 80 were purchased from Fisher Chemical (Fair Lawn, NJ, USA). $MgSO_4$ was ACS grade and purchased from J. T. Baker Chemical (Phillipsburg, NJ, USA). Potato dextrose agar (PDA) was purchased from Millipore (Darmstadt, DE).

Determination of parameters for AOP by model solution

AF stock solutions were prepared according to the AOAC official method 971.22 (AOAC, 2000). Hydrogen peroxide was diluted to obtain solutions with concentrations 1, 0.1, 0.01, 0.001, and 0.0001 g/hg. Diluted H_2O_2 solution (5 mL) was pipetted into a glass petri dish (Pyrex 55 mm × 17 mm, Corning, Glendale, AZ, USA) and mixed with 100 µL of AF stock solution containing 500 ng AFB1, 125 ng AFB2, 250 ng AFG1, and 125 ng AFG2. After adding AF stock solution, the mixture was immediately irradiated with 2.18 mW/cm^2 of radiation from two low-pressure (LP) mercury UV-C lamps (17 W, Model GPH357T5L/4P, LightSources Inc., Orange, CT, US) for 5 min to determine the appropriate concentration for AOP. The control group was prepared by adding

the same amount of AF stock solution into the same concentration of H_2O_2 solution and then quantitated by the AF assay immediately.

To determine the effective UV wavelength range, three types of LP UV lamps (17 W, Model GPH357T5L/4P, LightSources Inc., Orange, CT, US) emitting spectrums in UV-A, UV-B, and UV-C range and a customized 2000 W medium-pressure (MP) mercury UV lamp (LightSources Inc.) emitting a continuous spectrum from UV-C to the infrared range were used. The spectrums from four types of UV lamps used in this study were obtained by a miniature spectrometer (Model C-25, StellarNet, Tampa, FL, USA) and are shown in Fig. 7.1. Two LP UV lamps were held at 10 cm above the sample to deliver radiation with intensity at 2.18 mW/cm^2 (using LP UV-C lamp as standard). An MP UV lamp was held at the same height to deliver 12.48 mW/cm^2 of radiation. A petri dish loaded with AF model solution prepared as aforementioned was placed under UV lamps and irradiated for 3 and 6 min for LP lamps, or 30 and 60 s for MP lamp. A shorter irradiation time when applying the MP lamp was to adjust the intensity of the radiation to the same order of magnitude for comparing the AF degradation efficiency. AF concentrations were determined by the AF assay immediately after treatment. The experiments in this section were done in duplicate.

Evaluation of the effect of AOP on aflatoxin degradation in artificially spiked peanuts

Shelled, fresh runner-type peanuts (*Arachis hypogaea* L.) with less than 5 ng/g of AF were obtained from a local manufacturer (Golden Peanut Corporation, Alpharetta, GA, USA) and stored at 4˚C until used. To maintain the concentration of H_2O_2 during the AOP treatment, peroxidase and catalase were inactivated by roasting peanuts at 140 ± 5˚C for 10 min in a convection oven (Model MC32ACG-CHSS, Toshiba, Tokyo, JP). Roasted peanuts were spiked with AF stock solution to obtain a sample containing 100 ng/g AFB1, 25 ng/g AFB2, 50 ng/g AFG1, and 25 ng/g AFG2 by the method described in Shen & Singh (2021).

Spiked peanuts (100 g) and H_2O_2 solution (75 mL, 1 g/hg) were loaded in the UV equipment used in Shen & Singh (2021) for uniformly receiving UV radiation. The drum of the UV equipment was rotated at 11 rpm for 10 s followed by a stoppage of rotation for 350 s. The procedure was repeated 10 times (1 h) during the whole AOP treatment. Two LP UV-C lamps were held at 10 cm above the peanuts to deliver 2.76 mW/cm^2 of radiation. AF was quantitated by the AF assay. The experiments in this section were done in duplicate.

Verification of the effect of AOP in fungi inoculated peanuts

Peanuts were inoculated with *Aspergillus nomius* (NRRL 6108). The lyophilized spore pellet (0.1 g) received from Agricultural Research Service Culture Collection (Peoria, IL, USA) was dissolved in 2 mL sterile DI water (2 mL) to make the primary spore suspension. The suspension (0.1 mL) was pipetted and spread on PDA loaded in a petri dish (100 mm × 15 mm, VWR, Radnor, PA, USA) followed by being incubated at 30˚C, RH >90% for 4 days. The secondary spore suspension was prepared by rinsing Petri dishes with 10 mL of sterile 1 g/hg Tween 80 solution and then diluted to contain about 10^6 spores/mL. A batch of peanuts (1000g) was firstly rinsed with 500 mL of sterile DI water and drained. A 40 mL of the secondary spore suspension was added into the rinsed peanuts in a food storage box (28 cm × 18 cm × 8 cm, Wal-Mart Stores Inc., Bentonville, AR, USA). The box was mildly shaken until the liquid was completely absorbed and then covered with a surgical mask for fungi respiration. The peanuts containing spores were then incubated at 30˚C, RH > 90% for 6 days.

Inoculated peanuts were roasted at 140 ± 5˚C in the convection oven (Model MC32ACG-CHSS, Toshiba, Tokyo, JP) for 10 min to inactivate peroxidase and catalase. Roasted peanut kernels (100 g) were loaded into the drum of the UV equipment described in Section “Evaluation of the effect of AOP on aflatoxin degradation in artificially spiked peanuts.” A 75 mL of 10 g/hg

H_2O_2 was added to the drum loaded with kernels. The loaded kernels and H_2O_2 were treated with 1 h of UV-C irradiation (2.76 mW/cm^2). The rotation pattern was the same as described above. AF analysis by AF assay was done immediately after AOP treatment.

To further investigate the influence of enzymes on AF reduction by AOP, inoculated peanut kernels were roasted (140 ± 5˚C, 10 min) and then milled into particles about 1 mm in diameter by a blender (Model WF2211214, Waring corporation, Torrington, CT, USA). A 10 mL of 1 g/hg H_2O_2 was added into a petri dish (Pyrex 100 mm × 20 mm, Corning, Glendale, AZ, USA) loaded with 7.5 g of milled kernels were spread in a single layer and then treated with two LP UV-C lamps at an intensity of 2.18 mW/cm^2 for 1 h. AF analysis was done immediately after AOP treatment. The experiments in this section were done in triplicate.

Aflatoxin assay using immunoaffinity column and HPLC-Fluorescence detector (HPLC-FLD)

AF concentration was determined by the modified method AOAC 991.31 (AOAC, 2002) and normal phase HPLC-FLD (Leitao et al., 1988; Manabe et al., 1978). AF was extracted from about 25 g peanuts (weight was recorded to the second decimal) by blending with 125 mL of 70 mL/dL methanol and 5 g NaCl in a blender (Model WF2211214, Waring corporation, Torrington, CT, USA) at high speed for 2 min. The extract was filtered by a 24 cm fluted filter paper (Fisher Scientific, Rockingham, NH, USA). The filtrate (12 mL) was diluted with 20 mL of DI water. The diluted filtrate was filtered by a 1.5 µm glass microfiber filter paper (Whatman® 934-AH, GE Healthcare, Chicago, IL, USA). The secondary filtrate (10 mL for spiked peanuts, 2 mL for inoculated peanuts) was pipetted into an AflaTest® P immunoaffinity column (Vicam, Milford, MA, USA) and eluted at a rate of about a drop per second with an air pump. After elution, 20 mL of DI water was passed through the immunoaffinity column with the same rate for column washing. For liquid samples from Section “Determination of parameters for AOP by model solution,” one

mL of model solution was eluted by the immunoaffinity column followed by washing with 10 mL of DI water at the same rate. A 0.45 mL of acetonitrile was then passed through the immunoaffinity column by gravity to eluate the AF into a cuvette. Toluene (0.30 mL) and $MgSO_4$ (0.2g) were then added to the cuvette. The mixture was vortexed until the $MgSO_4$ particles aggregated and the solution became clear. The clear solution was pipetted into a two mL HPLC vial with a 0.4 mL insert (Fisher Scientific, Rockingham, NH, USA) for later analysis.

Liquid chromatography was done on an HPLC-FLD (Model Infinity 1260, Agilent, Santa Clara, CA, USA). A silica column (LC-Si, 5 µm, 25 cm × 4.6 mm, Supelco, Bellefonte, PA, USA) was used to separate AF. Before and after the analysis, the column was flushed with toluene/ethyl acetate (90 mL/10 mL) at a rate of 1.0 mL per min for 1 h. The column was held at 30˚C, flushed with the mobile phase at a flow rate of 1.5 mL/min. The mobile phase was prepared by mixing solution A (toluene/ethyl acetate/formic acid, 45/3/2, mL/mL/mL) and solution B (toluene/ethyl acetate/methanol, 45/3/2, mL/mL/mL) at a ratio of 1:1 (mL:mL) in the HPLC pump. Solution A and B can be stored at room temperature in the dark for up to two weeks. The sample injection volume was 40 µL. The excitation and emission wavelengths of FLD were 365 and 425 nm, respectively, with a sampling rate of 2.31 Hz. The concentration was determined by integrating areas under peaks. The AF concentration in the sample can be obtained by the following equations:

$$W = S\ g \times (12\ mL/125\ mL) \times (I\ mL/32\ mL) \quad (1)$$

$$E = E_{MeCN} + E_{Tol} = 0.75\ mL \quad (2)$$

$$\text{Aflatoxin in peanut (ng/g)} = (A \times E) / W \quad (3)$$

where W = corresponding weight of the sample to the eluate (g), S = dry weight of the peanut sample (g), which was obtained by subtracting the weight of increased moisture from the weight of the sample taken after treatment, I = volume of the secondary filtrate passed through the

immunoaffinity column (mL), E = volume of acetonitrile to elute aflatoxin from the IAC and toluene to dilute the eluate (mL), and A = concentration of aflatoxin in the eluate injected into HPLC (ng/mL). Total AF was obtained by adding concentrations of AFB1, AFB2, AFG1, and AFG2.

Oil quality evaluation using differential scanning calorimetry (DSC)

Oxidation induction time (OIT) was determined by the modified DSC method to evaluate oil quality changes after AOP treatment (Zhang et al., 2021). Fresh peanuts were roasted, AOP treated, or milled as described in Sections "Evaluation of the effect of AOP on aflatoxin degradation in artificially spiked peanuts" and "Verification of the effect of AOP in fungi inoculated peanuts." AOP-treated samples were further dried in the convection oven at 40 ± 5˚C for 12 h before OIT determination. Dried samples were then subjected to cold press (Model 3853, Carver Inc., Wabash, IN, US) to obtain oil samples. An aluminum crucible pan (40μL, Model ME-27331, Mettler Toledo, Columbus, OH, USA) was used to load 20.0 ± 0.5 mg of an oil sample. The pan without covering lid was placed into a DSC (Model DSC-1 700, Mettler Toledo, Columbus, OH, USA) previously stabilized at 25˚C and purged with 50 mL/min of N_2. The temperature of DSC was increased from 25°C to 140°C at a rate of 40°C/min and then held for 3 min for stabilization. After 3 min of stabilization, the purging gas was switched to O_2 (50 mL/min) to start an oxidation process. An exothermic peak on the isotherm was observed when the oxidation occurred. The OIT was determined by calculating the interception point (time) of the extrapolated baseline and the tangent line of the leading edge at the exothermic peak.

Hydrogen peroxide assay

Hydrogen peroxide concentration was determined by the modified method (Walsh et al., 2018). Peanut samples were prepared and dried as described in Section "Oil quality evaluation

using differential scanning calorimetry (DSC)." The weight and H_2O_2 concentration changes before and after drying were recorded. About 10 g of peanuts was sampled, mixed with 100 g of DI water, and mildly stirred for 1 min. A 10 g of the clear rinsed water was pipetted into an Erlenmeyer flask followed by adding 1 mL of 4.5 M of sulfuric acid, 1 mL of 1.5 g/dL ammonium molybdate, and 3 mL of 10 g/dL potassium iodide. The flask was continuously swirled during adding the reagents. After adding the reagents, the mixture was placed in the dark for 10 min. Before titration, 1 mL of 1 g/dL starch indicator was added into the flask. The mixture was then titrated with 0.05 M of sodium thiosulfate until the solution became clear. The control was prepared by adding 100 μL of 10 mg/mL catalase solution into the 10 g of rinsed water and then swirled for 10 min to decompose H_2O_2. After 10 min, the rinsed water was analyzed by the same procedure described above. The hydrogen peroxide concentration was determined by the following equation:

$$\Delta V = V_{sample} - V_{control} \quad (4)$$

$$H_2O_2 \text{ concentration (g/g)} = (\Delta V \times 1/1000 \times N/2 \times M_{H2O2}) / (W_{peanut} \times 10g/100g) \quad (5)$$

where V_{sample} = volume of sodium thiosulfate for the titrating sample solution (mL), $V_{control}$ = volume of sodium thiosulfate for titrating the control solution (mL), N = normality of sodium thiosulfate (mol/L), M_{H2O2} = molar mass of H_2O_2 (g/mol), and W_{peanut} = dry weight of the peanut sample (g), which was obtained by subtracting the weight of increased moisture from the weight of the sample taken after treatment.

Color measurement

Color of peanuts (CIELAB color space) was measured using a colorimeter (Model MiniScan EZ 4500L, HunterLab, Reston, VA, USA) to take the average L* a* b* value from three different positions in the same batch of peanuts.

Statistical analysis

ANOVA table and Tukey's pair test were performed on JMP Pro 15.0.0 software (SAS Institute Inc., Cary, NC, USA). The significant level was set at 0.05.

Results and discussion

Determination of parameters for AOP

In the experiment treating AF model solution added with different concentrations of H_2O_2 from 0.0001 g/hg to 1 g/hg, we found that the presence of H_2O_2 with a concentration higher than 0.01 g/hg largely increased the AF reduction. When the concentration of H_2O_2 was lower than 0.01 g/hg, the reduction levels were not significantly different from the solution containing only water. No AF was detected when the H_2O_2 concentration was higher than 0.1 g/hg with LP UV-C lamp treatment. The use of H_2O_2 in AOP with a concentration that must be higher than 0.01 g/hg could be due to the low molar extinction coefficient of H_2O_2, which is 19.6 $M^{-1}cm^{-1}$, under 254 nm of radiation (Glaze et al., 1987). As the main absorber in AOP, H_2O_2 concentration must exceed 0.01 g/hg to produce enough hydroxyl radicals for efficient AF decontamination.

The addition of H_2O_2 not only promoted AF degradation but also assisted in decomposing the degradation products from AF, such as aflatoxin B2a (AFB2a). As shown in Fig. 7.2, we found that AFB2a was not observed in the HPLC chromatograph in the model solution subjected to the AOP. AFB2a is a derivative from AFB1 produced by UV-C irradiation. The compound has about 200 times lower toxicity and a much higher fluorescence response than AFB1 (Shen & Singh, 2021a). AFB2a presented in the chromatograph even though the sample was cleaned up by immunoaffinity column. This could result from the high similarity of the structure between AFB2a and AFB1. This finding implies that quantitating AF by only a fluorometer (or "bromine solution method") described in AOAC official method 991.31 may overestimate the concentration of AF in

the sample subjected to UV radiation (AOAC, 2002). We had found that samples treated with UV showed a higher AF concentration when applying the bromine solution method.

The spectrums from four types of UV lamps used in this study are shown in Fig. 7.1. The maximum photon emission was at 365 nm for the LP UV-A lamp, 310 nm for the LP UV-B lamp, and 254 nm for the LP UV-C lamps, whereas the MP lamp emitted continuous, polychromatic radiation from 190 to the infrared range. Table 7.1 shows AF reductions in 0.01 g/hg H_2O_2 solutions irradiated with four types of UV lamps. Both LP UV-A and LP UV-C lamps demonstrate the highest AF reduction. The result confirmed our expectation that H_2O_2 has the highest absorption in the UV-C range, implying that more free radicals can be generated with the radiation in that range (Lin et al., 1978). UV-C is conventionally selected as the radiation source of AOP for the same reason. Additionally, AF shows a maximum absorption of around 365 nm, indicating that it may act as a photosensitizer (Stanley et al., 2020). The result also accords with Yousef & Marth (1986) who observed a 72% of aflatoxin M1 reduction by treating contaminated milk with UV-A lamps and 0.05 g/hg of H_2O_2 for 25 min. Even though UV-C and UV-A ranges have the same level of AF reduction, the degradation pathway might be different. AF may be mostly decomposed by being oxidized by hydroxyl radicals generated by UV-C or being activated directly by UV-A radiation followed by reacting with other surrounded molecules such as water and H_2O_2. However, Bulman et al. (2019) reported that other wavelengths such as 311 and 365 nm also demonstrated an ability to generate free radicals, so the degradation of AF under UV-A irradiation may undergo both degradation pathways. The phenomenon is critical in AF detoxification application since we found that the OIT of peanut oil treated with UV-A was not significantly different from the oil without treatment, whereas the OIT was significantly shorter when the oil sample was irradiated

by UV-C radiation. UV-A radiation is in abundance in solar radiation, so the technology has the potential to be deployed in those areas that lack electricity.

Even though the MP lamp emitted radiation involving wavelengths at 254, 311, and 365 nm, the AF decomposition efficiency was not as good as the LP UV-C lamp. The MP lamp showed almost the same level of AF reduction as LP UV-C did when the delivered radiation dose was adjusted to the same order of magnitude. However, the MP lamp consumed about 59 times higher energy than two LP UV-C lamps. Most of the energy is wasted on emitting radiation in visible and infrared ranges, which contributes much less to AF degradation. Accordingly, we still used LP UV-C lamp in the following experiments in this study because using UV-C as the radiation source of AOP has been well studied.

Evaluation AOP effect in spiked peanuts

In our observation, H_2O_2 was catalyzed into oxygen gas and water when treating peanut samples with AOP. Enzymes like catalases or peroxidases are widely spread in the biological organism as a scavenger to remove superoxide compounds generated from aerobic metabolism (Imlay, 2003). These enzymes can quickly lower the H_2O_2 concentration and hence impede the AOP. As a result, we enhanced the concentration of H_2O_2 from 0.01 g/hg to 1 g/hg in this experiment. Additionally, we compared the peanuts with and without roasting to evaluate the influence of the enzymes. The peanuts were roasted at 140 ± 5˚C for 10 min producing almost no bubble when immersing in the 1 g/hg H_2O_2 solution for 10 min.

As can be seen in Table 7.2, enzyme inactivation by roasting slightly influences AF reduction. There is no significant difference in AF reduction between the peanuts with and without roasting, which could result from the incomplete inactivation of the catalase in the interior part of peanut kernels. We found that after 1 h of AOP, bubbles of generated oxygen gas came out from

the inside of roasted peanut kernels, whereas no bubble was observed at the initial stage of AOP. A smaller standard deviation of the AF reduction in roasted peanuts than those un-roasted was observed. The inherently different catalase distribution in each kernel may contribute to the most of variation of AF concentration change. Therefore, less enzyme activity in peanuts may result in a more uniform AF concentration change before and after AOP treatment. That is, for fresh peanut kernels, a greater variation of AF concentration change was produced due to the higher concentration of enzymes in peanut kernels. Therefore, we still used roasted peanuts for the remainder of the experiments. Methods for inactivating catalase or other radical scavenger systems in the treated foods should be further investigated.

Employing AOP in AF decontamination is more efficient than using UV only. In our previous study, AFB1 reduced 18% with the same UV equipment and similar processing parameters for two hours (Shen & Singh, 2021b). Additionally, the AFB1 reduction was "saturated" when longer than 2 h of irradiation was employed. That could be because the UV radiation cannot penetrate the deeper part of kernels to decompose AF. With the addition of H_2O_2, the AFB1 reduction was up to 34% in the 1 h treatment, which is almost two times higher than that of the 2 h UV-only treatment. The enhanced AFB1 reduction might be contributed from not only the promotion of AFB1 degradation by hydroxyl radicals from AOP but also the better penetrability of the AOP. Reducing more AF in contaminated peanuts is possible with a prolonged treatment time. Nonetheless, as mentioned before, methods for maintaining H_2O_2 concentration at a certain level for performing AOP should be introduced. For example, resupplying H_2O_2 for every certain of period time during the AOP treatment would be useful.

Verifying AOP effect in inoculated peanuts

It is very difficult to evaluate the AOP effect on decontamination of fungus inoculated peanuts as long as the AF is unevenly distributed in the sample. The relative standard deviation of total AF contain in inoculated peanuts is almost one-third, even though the peanuts were carefully prepared under the laboratory condition (Table 7.3). The heterogeneous distribution of AF in peanuts behaves the instinct of fungus growth, which is not evenly distributed on the whole batch of peanuts. The phenomenon can also be observed in either naturally occurred or artificially inoculated contaminations (Martins et al., 2017; Van Egmond & Jonker, 2004). To overcome the issue, we raised the H_2O_2 concentration to 10 g/hg and prolonged the treatment time to 2 h. However, as shown in Table 7.3, there is still no significant difference in AF concentration changes between the sample before and after treatment. Therefore, it is hard to conclude whether the observed reduction was caused by the AOP treatment or the uneven AF distribution. The phenomenon also reveals a fact that the AF may distribute mainly in the interior of kernels where AOP treatment can hardly reach. It seems like the fungus has evolved to give AF great mobility to migrate inside the seeds or plant bodies as a phytotoxin (Cucullu et al., 1966; Klich, 2007; McLean et al., 1994). This finding elucidates using fungus contaminated peanuts for verifying a newly proposed AF decontamination process is necessary.

We further milled the peanuts into small particles about 1 mm in diameter to mitigate the issue. As shown in Table 7.3, milling considerably lowered the standard deviation of AF concentrations. More importantly, milling significantly enhanced the total AF reduction to about 60% in an hour. The concentration of H_2O_2 solution was lowered to 1 g/hg because we found that foam was vigorously generated and spilled over from the petri dish when H_2O_2 solution with a higher concentration was added into milled peanut particles even the peanut had been roasted. The foam was also formed with the addition of 1 g/hg H_2O_2 solution, but it only formed a 10-mm thick

layer and covered the peanut particles. The influence of the foam layer on the AF degradation efficiency is not clear. The foam layer could absorb the UV radiation and lower the AF decomposing by AOP. Moreover, in our observation, the inoculated peanuts seemed to produce more foam, compared with those uncontaminated peanuts. Clavero et al. (1993) thought that the catalase activity was increased because of the growth of Aspergillus. Increased catalase can generate more bubbles in the H_2O_2 solution and can be used to separate peanut kernels infected with fungus from healthy ones. Therefore, developing a method to inactivate catalase activity in peanuts while performing AOP could improve the AF detoxification efficiency.

Evaluation of the quality changes after AOP treatment

For whole peanut kernels, AOP had only a few effects on quality. The oil quality was determined by the DSC method rather than total oxidation value (TOTOX) since we found that TOTOX is not sensitive enough to detect small oil quality changes. The TOTOX of oil in peanuts did not significantly change after AOP treatment, but the peanuts had a very light rancidity smell. Table 7.4 shows the quality changes of peanuts before and after AOP treatment. The OIT of peanuts after AOP treatment was slightly shortened, indicating the oil quality had not deteriorated. We expected a significant oil deterioration due to high unsaturated fatty acids in peanuts oil. The color of AOP treated peanuts became darker according to the lower L* value, but it was not obvious by visual inspection. This could be due to the oxidation of phenolic compounds which are rich in peanut skin. No H_2O_2 was detected in AOP treated peanuts after the subsequent drying process indicating that the process met the standards of the US FDA. The weight of peanuts before and after the AF detoxification process had a very small change, implying that no significant mass transfer occurred. Nonetheless, details in nutrient changes such as protein or starch should be further investigated. The process of catalase inactivation and H_2O_2 treatment was similar to the

blanching step to remove the skin of peanuts in peanut processing. Therefore, the proposed conditions can be combined with the current processing steps applied in the peanut industry.

It was difficult to evaluate quality changes in milled kernels before and after AOP treatment. The foam layer combined with peanut particles formed a "cake-like" solid after AOP and a drying process, and thus measuring the residual H_2O_2 as well as the weight change became more complicated. The color change could be measured, but it might be meaningless because the sample had lost its original appearance completely after the AOP treatment. Eventually, we only measured the OIT. Even though milling significantly improved the AF reduction, the oil quality was seriously affected. It seems that treating milled peanuts with AOP is not a suitable method to reduce AF while retaining the quality and appearance. Nonetheless, the produced "cake" can be used as a material for processed foods or animal feeds. Alternatively, using UV-A as the radiation source could be another way to maintain the oil quality after AOP as mentioned in Section "Determination of parameters for AOP."

Conclusions

This study shows that the combination of UV and H_2O_2 not only accelerated the degradation rate of AF but also was able to degrade the degradation compound. Both the LP UV-A and LP UV-C lamps can be used as the radiation source for AOP. The radiation in the UV-C range is mostly used in AOP, yet it may cause oil deterioration. Using radiation in the UV-A range to reduce AF while maintaining the oil quality of peanuts is possible and needs further study. Maintaining the concentration of H_2O_2 by inactivating enzyme activities or other possible means during AOP should be improved to enhance the AF degradation efficiency. Means to utilize the milled peanuts with quality and appearance changes after AOP treatment should be considered.

The AOP treatment can be considered environmentally friendly. We demonstrated that even UV lamps with low energy consumption (34 W) were enough to reduce AF. Additionally, the reagent, H_2O_2, can be easily removed or degraded by evaporation or enzyme catalysis. The degradation compounds are only water and oxygen. Further, no detectable H_2O_2 was observed in peanuts after AOP treatment, which justified the use of AOP in decontaminating AF or even other kinds of contaminations.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table

Table 7.1 Aflatoxin concentration in model solution containing 0.01 g/hg of H_2O_2 before and after being irradiated with four types of UV lamps (n = 2).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1*	AFB2*	AFG1*	AFG2*	Total AF*§
Control	113.38 ± 10.55^{a}	31.46 ± 10.28^{a}	51.77 ± 7.23^{a}	23.88 ± 2.11^{a}	220.50 ± 30.17^{a}
LP UV-A†					
3 min	38.82 ± 3.07^{d}	1.62 ± 0.08^{bc}	33.46 ± 0.49^{bc}	8.58 ± 0.02^{d}	82.48 ± 3.50^{c}
6 min	17.23 ± 0.48^{de}	0.07 ± 0.10^{c}	24.11 ± 2.28^{cd}	3.85 ± 0.66^{e}	45.25 ± 2.55^{cd}
LP UV-B†					
3 min	93.26 ± 8.96^{ab}	18.23 ± 4.53^{abc}	51.40 ± 5.85^{a}	22.32 ± 1.27^{ab}	185.20 ± 11.55^{ab}
6 min	73.97 ± 7.46^{bc}	10.99 ± 1.70^{bc}	49.07 ± 4.77^{a}	19.24 ± 1.12^{bc}	153.28 ± 11.64^{b}
LP UV-C†					
3 min	26.38 ± 5.52^{de}	5.26 ± 1.40^{bc}	16.14 ± 3.46^{de}	8.47 ± 0.62^{d}	56.25 ± 11.00^{cd}
6 min	8.22 ± 1.69^{e}	1.17 ± 0.73^{bc}	6.30 ± 0.61^{e}	4.24 ± 0.04^{e}	19.94 ± 2.98^{d}
MP†					
30 s	67.03 ± 1.07^{c}	19.14 ± 7.00^{ab}	39.56 ± 1.77^{ab}	22.35 ± 0.22^{ab}	148.07 ± 4.38^{b}
60 s	39.80 ± 1.67^{d}	9.54 ± 3.98^{bc}	26.35 ± 2.01^{bcd}	17.43 ± 0.64^{c}	93.12 ± 0.94^{c}

* AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

§ The sum concentration of AFB1, AFB2, AFG1, and AFG2.

† LP: low-pressure lamp, MP: medium-pressure lamp.

$^{a, b, c, d, e}$ letters represent the significant difference ($p < 0.05$) within the same column.

Table 7.2 Aflatoxin concentrations in artificially AF spiked peanuts (with and without roasting) before and after AOP treatment (n = 2).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1[*]	AFB2[*]	AFG1[*]	AFG2[*]	Total AF[*§]
Raw peanuts					
control	93.67 ± 2.21	24.72 ± 0.72	47.12 ± 0.29	14.23 ± 2.64	179.74 ± 0.87
AOP, 1 h[†]	64.07 ± 6.03	16.91±1.49	31.04 ± 1.90	7.67 ± 2.97	119.70 ± 12.39
Reduction (%)	32 ± 5[a]	31 ± 8[a]	34 ± 4[a]	43 ± 31[a]	33 ± 5[a]
Roasted peanuts					
control	99.52 ± 0.30	27.05 ± 0.28	50.33 ± 0.38	16.04 ± 1.87	192.95 ± 2.26
AOP, 1 h[†]	65.20 ± 0.76	18.65 ± 0.48	33.26 ± 0.40	11.76 ± 1.62	128.86 ± 3.26
Reduction (%)	34 ± 1[a]	31 ± 2[a]	34 ± 0[a]	27 ± 2[a]	33 ± 1[a]

[*] AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

[§] The sum concentration of AFB1, AFB2, AFG1, and AFG2.

[†] AOP: advanced oxidation process (100 g of sample was immersed in 75 mL 1 g/hg H_2O_2 and irradiated with 2.76 mW/cm^2 of radiation from two low-pressure UV-C lamps).

[a] letter represents the significant difference ($p < 0.05$) of AF reduction within the same column.

Table 7.3 Aflatoxin concentration changes of the inoculated whole peeled kernel and milled peeled kernel before and after treated by advanced oxidation process (2.18 mW/cm^2 of low-pressure UV-C lamps,10 g/hg H_2O_2 for the whole kernel, and 1 g/hg for milled kernel) (n = 3).

Treatment	Aflatoxin concentration (ng/g)				
	AFB1[*]	AFB2[*]	AFG1[*]	AFG2[*]	Total AF[*§]
Whole kernel					
Control	1572.78 ± 823.83	35.71 ± 18.89	1363.02 ± 465.23	13.89 ± 16.73	2985.40 ± 861.38
AOP (10 g/hg H_2O_2)[†], 1 h	1093.28 ± 851.71	52.75 ± 41.25	0.85 ± 0.01	48.91 ± 36.31	1195.79 ± 929.11
AOP (10 g/hg H_2O_2)[†], 2 h	678.01 ± 664.44	31.19 ± 25.19	1757.36 ± 1355.79	33.66 ± 28.75	2500.22 ± 1972.74
p-value	0.429	0.668	0.089	0.379	0.316
Milled kernel					
Control	1007.98 ± 362.47	44.14 ± 18.72	1235.63 ± 428.70	31.01 ± 14.36	2318.76 ± 817.55
AOP (1 g/hg H_2O_2)[†], 1 h	372.34 ± 4.41	16.39 ± 0.23	525.19 ± 35.35	17.84 ± 0.93	931.76 ± 31.98
p-value	0.039	0.062	0.046	0.188	0.043

[*] AFB1: aflatoxin B1, AFB2: aflatoxin B2, AFG1: aflatoxin G1, AFG2: aflatoxin G2, Total AF: total aflatoxins.

[§] The sum concentration of AFB1, AFB2, AFG1, and AFG2.

[†] AOP: advanced oxidation process (100 g of sample was immersed in 75 mL H_2O_2 and irradiated with 2.76 mW/cm^2 of radiation from two low-pressure UV-C lamps).

Table 7.4 Peanuts' quality changes after being roasted at 140˚C for 10 min, treated by AOP for 1 h, and dried at 35˚C for 12 h (n = 3).

Treatment	Residual H_2O_2 (g/hg)	Weight (g)	OIT[§] (min)	Color (CIELAB color space)		
				L^*	a^*	b^*
Whole kernel						
Roasting	-	100.90 ± 0.57	61.6 ± 4.7	45.25 ± 0.45	14.51 ± 0.35	24.65 ± 1.76
AOP[†], 1 h	0.312 ± 0.084	122.85 ± 0.78	-	-	-	-
Drying	n.d.[¶]	102.90 ± 0.10	52.3 ± 5.8	43.64 ± 0.21	15.09 ± 0.27	27.23 ± 0.64
p-value	-	-	0.221	0.044	0.206	0.190
Milled kernel						
AOP[‡], 1 h	-	-	-	-	-	-
Drying	-	7.5 ± 0.0	24.3 ± 6.0	-	-	-

[§] OIT: oxidation induction time.

[†] AOP: advanced oxidation process (100 g of sample was immersed in 75 mL 10 g/hg H_2O_2 and irradiated with 2.76 mW/cm^2 of radiation from two low-pressure UV-C lamps).

[‡] AOP: advanced oxidation process (7.5 g of sample was immersed in 10 mL 1 g/hg H_2O_2 and irradiated with 2.18 mW/cm^2 of radiation from two low-pressure UV-C lamps).

[¶] Not detectable.

Figures

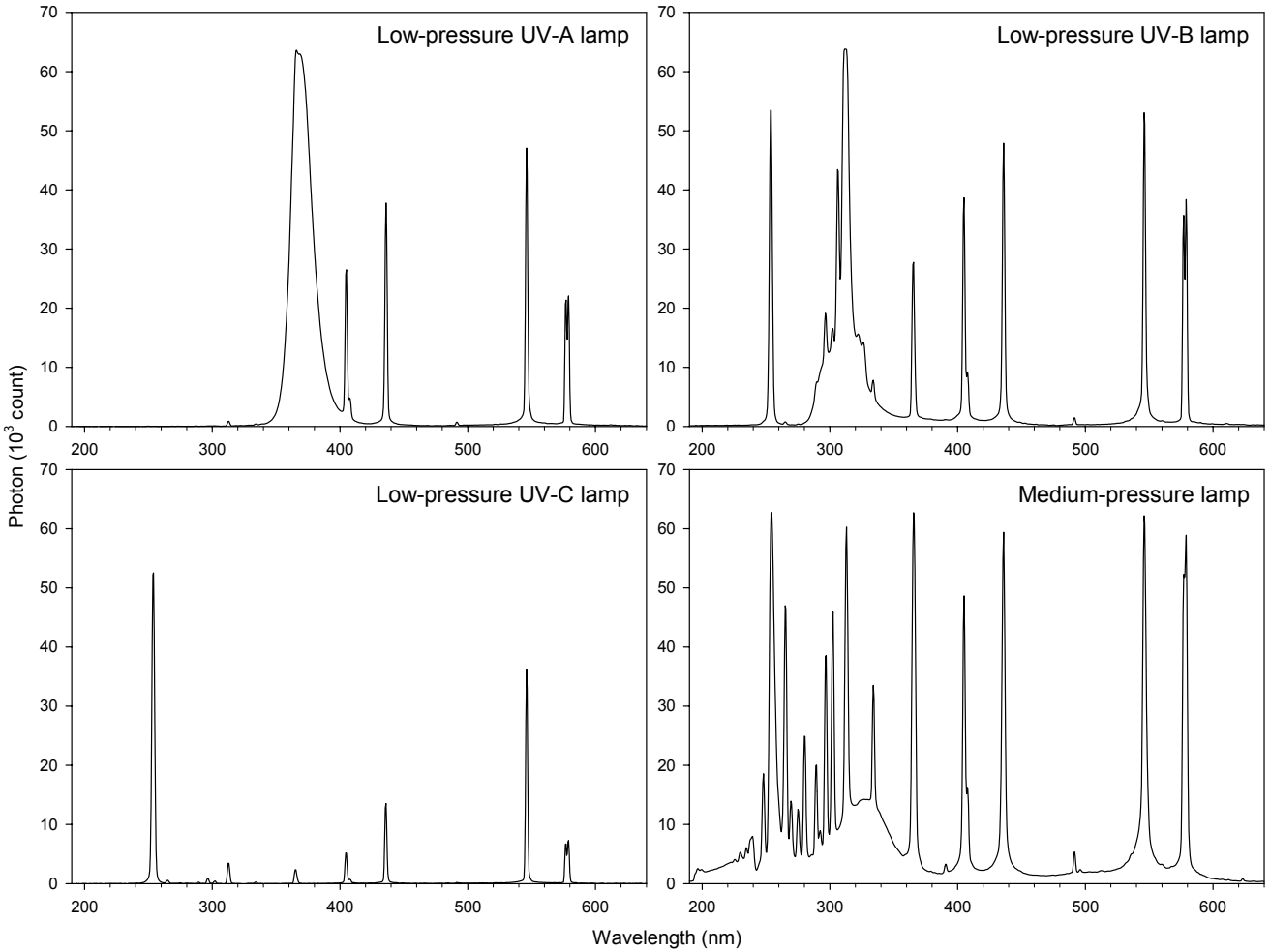

Figure 7.1 Emission spectrums of the three low-pressure UV lamps and the medium-pressure UV lamp used in this study (intensities were adjusted to the same scale).

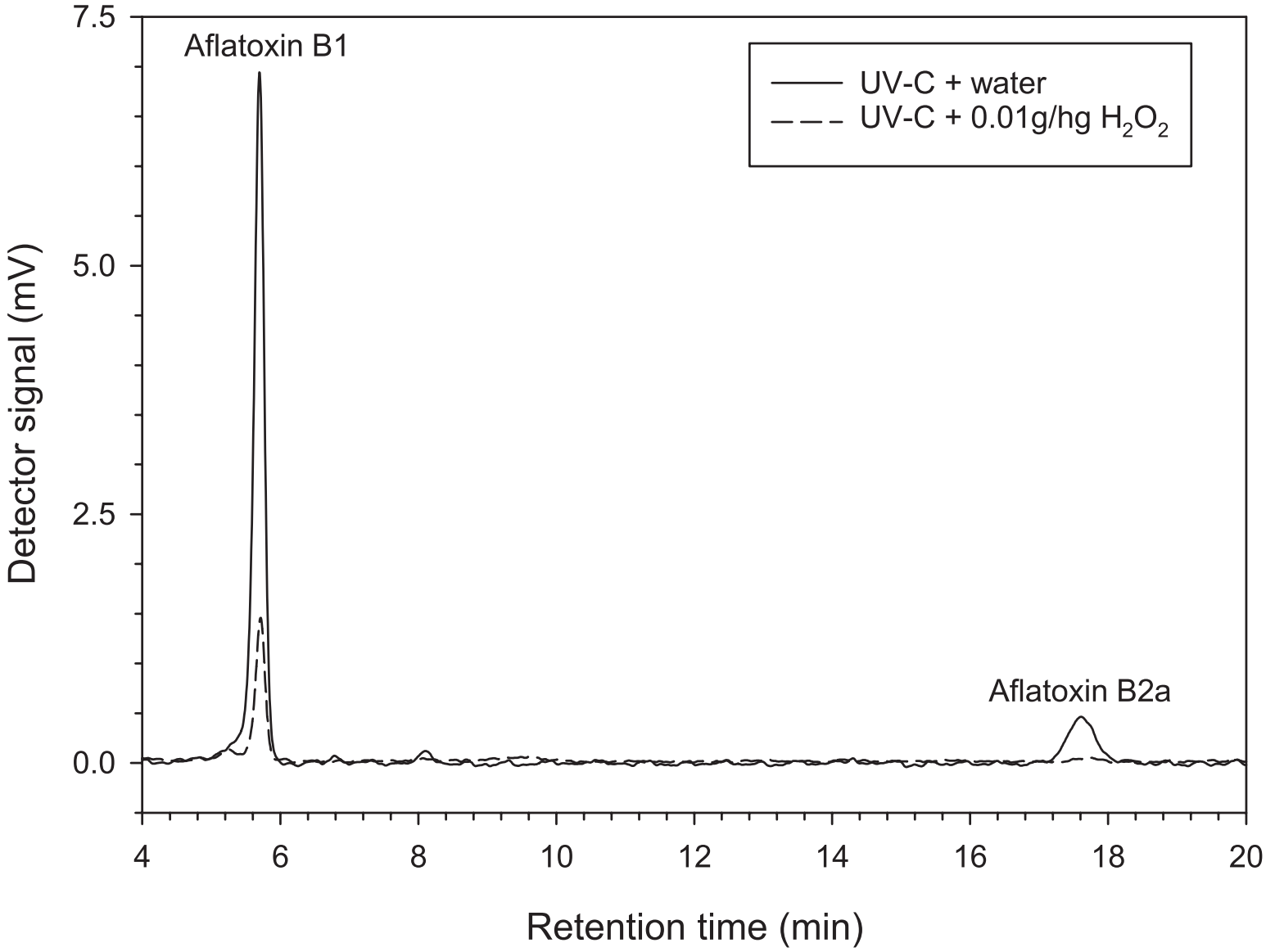

Figure 7.2 Aflatoxin B2a observed in HPLC chromatogram of the model solution treated with UV-C (2.18 mW/cm^2) and water.

CHAPTER 8

CONCLUSIONS & FUTURE RECOMMENDATIONS

Many aflatoxin detoxification methods have been proposed to deal with more and more serious contamination issues caused by global warming. Most methods demonstrate a great aflatoxin reduction but may not be practical to be used in the food industry due to the availability or the cost of the technics. We envision that this study can make significant progress in using UV and H_2O_2 to reduce aflatoxin in contaminated foods. The complicated aflatoxin analysis procedure was also shortened and more efficient according to our results. The proposed works are the extension of conventional UV technology but overcome its disadvantages such as slow aflatoxin reduction rate, uneven radiation of the process, or oil deterioration of oily samples.

All the objectives in this study are addressed. The performance of the modified AF analysis was greater than the original one after we replaced the laborious and time-consuming derivatization procedure with the normal-phase condition. The LOQ of the method fulfills the requirement of the US FDA and has the potential to achieve stricter standards. The proposed method was applied in this study to determine AF concentration in the sample. The uniformity of UV radiation was evaluated by coating peanuts with AgCl photography emulsion as a radiation dosimeter. By rotating peanuts within a stainless steel treatment chamber at 11 rpm, the radiation was uniformly received by peanuts, resulting in a 25% more reduction of AF. UV-A radiation was found to be more effective to degrade AF than UV-C. Oil in peanuts was almost not affected by the UV-A radiation but was significantly deteriorated after exposure to UV-C radiation. We also found that milling whole peanut kernels into small particles may be a critical step to completely

decontaminate AF since part of AF was located deep inside the peanut where UV cannot arrive. Mildly increasing temperature to 50˚C largely increased the AF decomposition rate in H_2O_2 solution but retained the quality of peanuts. Inactivation of H_2O_2 catalysis enzymes can enhance the AF reduction, but the mechanism needs to be further investigated. It seems that the presence of H_2O_2 catalysis enzymes in high-concentration H_2O_2 solution can promote the degradation of AF under certain conditions. The residual H_2O_2 in peanuts can be easily removed by air drying, so the safety concern should be limited. The proposed advanced oxidation processes were able to reduce up to 60% of aflatoxins in contaminated peanuts within an hour, which was faster than using UV or H_2O_2 treatment alone. The oil quality was slightly affected after the combination treatment but acceptable. Using UV-C radiation was most effective to degrade AF, compared to UV-A and UV-B. Breaking down peanuts into smaller particles can promote AF decomposition and hence should be used in a detoxification process.

These treatments are especially suitable to be deployed in those areas where contaminations frequently occur due to the lack of infrastructure or electricity for UV equipment, such as most developing countries. This study showed that solar radiation can be used as the UV source, so no electricity is needed. H_2O_2 is also an affordable and easily available chemical for the decontamination process. On the other hands, treatments like roasting, peeling, or treating with H_2O_2 can be involved in the conventional peanut processing operations such as blanching. For instance, the roasting process followed by H_2O_2 treatment considerably loosen the skin, facilitating peanut skin to be removed mechanically. That is, we believe that the proposed treatments can assist in solving the aflatoxin contamination that occurred around the world with almost no additional cost. Additionally, these treatments only need minimum equipment and energy to perform and hence have very limited impacts on the environment, fulfilling the requirement of sustainability.

Future works may focus on:

(1) To optimize the parameters for the proposed normal-phase HPLC method to further lower the limit of quantification.

(2) To construct a more precise UV profile by introducing AI modeling for evaluating the radiation uniformity from the observed color change.

(3) To investigate the AF reduction efficiency and the change of UV-sensitive ingredients in other foods after UV-A treatment, such as the change of aflatoxin M1 in milk and its oil oxidation profile.

(4) To investigate how H_2O_2 catalysis enzymes such as peroxidase affect aflatoxin degradation in foods.

(5) To examine whether UV-A radiation can be used as an alternative UV source in advanced oxidation processes and its influence on UV-sensitive ingredients such as oil.

Aside from these points, the quality of nutrient in peanuts, the residual toxicity of AF after proposed treatments, and the sensory evaluation for peanuts after treatment, should be further investigated.