

INACTIVATION OF AFLATOXINS B₁, B₂ IN PEANUTS BY PULSED LIGHT (PL)

By

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LIST OF ABBREVIATIONS

AFTs	Aflatoxins
AFB ₁	Aflatoxin B ₁
AFB ₂	Aflatoxin B ₂
AFM ₁	Aflatoxin M ₁
AFG ₁	Aflatoxin G ₁
AFG ₂	AflatoxinG ₂
AOAC	Association of Analytical Chemists
AOCS	American Oil Chemists Society
ANOVA	Analysis of Variance
ESI	Electrospray Ionization
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food and Drugs Administration
FFA	Free Fatty Acids
FAO	Food and Agriculture Organization
KMC	Kernel Moisture Content
LSD	Least Significant Differences
LP	Low Pressure
meq	Milliequivalent
MP	Medium Pressure

PV	Peroxide Value
WHO	World Health Organization
SPSS	Statistical Package for the Social Sciences

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Aflatoxin(s) (AFTs) are secondary metabolites of *Aspergillus molds*. These toxigenic, carcinogenic, and immunosuppressive compounds are commonly found in contaminated peanuts. Most of the methods studied to eliminate AFTs on peanuts either degraded peanut quality or were not cost effective. Pulsed light (PL) treatment has shown promising results in AFT degradation in beverages and other food products. Five methods were tested using with- and without-skin peanut kernels that were with *A.falvus* and PL-treated using: 1) aluminum plate on a conventional conveyor, 2) shaker, 3) rotating glass tubes, or 4) on a plate with ice; additionally, sliced peanuts were tested on 5) aluminum plate on a conventional conveyor. The shaker treatment was further tested using a hurdle technique of soaking the peanuts in a citric acid solution prior to PL treatment. AFB₁ and AFB₂ contents were determined using an enzyme-linked immunosorbent assay (ELISA) and liquid chromatography mass spectrometry (LC-MS/MS). Finally, the effect of storage time on PL treated peanuts peroxide value and free fatty acids were investigated. Data were analyzed by Analysis of Variance (ANOVA) using SPSS and LSD at $\alpha=0.05$.

Peanuts treated on conveyer plate for 300 s at 5 cm from the strobe showed AFTs were reduced by 82% with-skin and 95.3% without-skin. However, the surfaces of the peanuts were burned. Surface burning was avoided by shaking or rotating the peanuts during the treatment. Shaking treatment had the significantly ($p < 0.05$) higher AFTs reduction (91.73%) compared to other methods, presumably due to exposing all peanut sides to the light during the treatment. The hurdle techniques achieved 100% reduction with 240s of PL treatment. Peroxide and free fatty acids values were not significantly increased after 3 months of storage. This study indicates that a cost effective PL technique can achieve significant reductions in AFTs that are superior to other methods without altering the quality of the peanuts.

CHAPTER 1 INTRODUCTION

Fungi or molds have played a paramount destruction role in agriculture commodities throughout history. They can contaminate and colonize crops before harvesting and during storage for a relatively long time in a warm and high-humidity condition or exposure to a stressful environment such as drought. Molds can excrete extremely harmful secondary metabolites called mycotoxins inside and over the crops. There are hundreds of mycotoxins; few have been detected in food and are considered to have a serious impact on human health (Serra et al., 2005).

Mycotoxins have been studied since the early nineteenth century (Sinha & Arora, 1911). Many fungi species are reported as mycotoxin producers; however, the filamentous fungi *Aspergillus* is the dominant producer. *Aspergillus* currently is one of the most economically distinguished genera due to its ability to produce aflatoxin(s) (AFTs). *Aspergillus* species produce AFTs during its living phase. However, the exact function of these mycotoxins remains a mystery. It is hypothesized that mycotoxins act as a defense mechanism, protecting the fungus from plants, animals, and other competing fungi (Smith and Moss, 1985). *Aspergillus flavus* and *Aspergillus parasiticus* are the main AFT producers. In addition, the gene responsible for producing these toxins doesn't exist in some other *Aspergillus* species such as the food fermentation useful strains *A. oryzae* and *A. sojae* (El-Nagerabi et al., 2012; Hassan et al., 2017; Kusumoto et al., 1998). The major AFT concern to human health is from four types of aflatoxins B₁, B₂, G₁, and G₂. The aflatoxin B series, especially B₁ is the most severe AFT. It has multifactorial toxic and chronic effects. Particularly those which are attributed to hepatocarcinogenic, hepatotoxic, teratogenic, and mutagenic effects

(Moreau et al., 2013; Moudgil et al., 2013), Major AFT infected commodities are dried crops such as oil seeds, cocoa, coffee, dried peas, spices, fruit, beans, and nuts, especially peanuts (Turner et al., 2009).

Peanuts (*Arachis hypogaea*) is commonly named worldwide as groundnut and earthnut, monkey-nuts, and goobers. It is one of the most nutritious crops and one of the world's most popular and consumable commodity, cultivating in approximately 100 countries in all six continents. Peanuts have grown originally in South America in tropical and subtropical regions. Peanuts are considered one of the most produced and consumed commodities in the United States for its high nutrient value. Peanuts contain 51.9% lipid, and 21% to 36% protein in total mass. The peanut's fatty acid composition contains approximately 43% oleic acid, 35% linoleic acid, and 0.1% linolenic acid. Peanut oil has a low vulnerability to oxidation because of the less double bonds present in the peanut's oil compared with other legumes and nuts (Wang et al., 2016; Zhao, 2013). In addition, peanuts are rich in Vitamin E and phytosterols which are related to cardiovascular and coronary heart disease's prevention, cancer reduction, and immune system fortification. Furthermore, Harvard University's Public Health Center demonstrated the consumption of peanuts or peanut butter could reduce the threat of developing type two diabetes (Eisenstat et al., 2007).

Peanuts are contaminated with AFTs when subjected to extended periods of pre-harvest heat and drought stress (Holbrook et al., 2000). Changes of chemical composition of peanut kernels due to fungal infection are inevitable. Proteins, lipids, free and total amino acids, and free fatty acids are reported to change significantly when peanuts are infected by *A. flavus* (Chiou et al., 1994).

Since AFTs have been discovered, several investigations have been conducted regarding detoxification and elimination methods. AFTs are notoriously difficult to remove. Most AFTs are chemically stable and heat resistant toxins. Thus, they tend to survive processing stages. AFTs tolerate the high temperatures encountered during baking, roasting, and breakfast cereal production. Most of the chemical, physical, microbial, and irradiation approaches that have been tested never reached complete AFT elimination. This makes it essential to prevent AFT production in the first place by avoiding the conditions which lead to mycotoxin formation, which may not always be achieved during practice (Turner et al., 2009). Additionally, any detoxification process to be applied practically, should be economically and technically feasible.

Pulsed ultra-violet light (PL) is one of the proposed new technologies which is presented as non-thermal food treatment regarding microbial inactivation by using broad spectral wavelengths (200 to 1100 nm), including the UV spectrum. PL has proven its capability of destroying and reducing harmful compounds, bacteria, viruses, enzymes, allergens, and toxins. This research proved the efficiency of PL to inactivate AFTs to significantly lower levels in peanut kernels.

Objectives. The overall purpose of this study was to investigate the effect of the pulsed light technique on AFTs in peanuts. To inactivate the AFTs totally or partially to meet the regulatory requirements (e.g. FDA, EU, etc.), the specific objectives that were investigated in this project are:

Objective 1: Determine the degradation percentage of aflatoxins (B₁, B₂) in without-skin (w/o the testa) and with-skin peanuts (with the testa), after PL treatment using different distances and times using an enzyme linked immunosorbent assay (ELISA) and liquid chromatography-mass spectrometry (LC-MS/MS) for quantification.

Objective 2: Optimize different conditions and treatments (in plate, in ice, in rotating tube, slices, and shaking treatment) using several distances 5, 7, and 10 cm, and different times of PL treatment.

Objective 3: To combine PL with citric acid and compare the results with the PL treatment alone.

Objective 4: Monitor the treated peanuts after storage by recording any possible changes in peroxide value, free fatty acids, and acid value as proof of lipid oxidation, after variant storage periods (1, 2, and 3 months).

CHAPTER 2 LITERATURE REVIEW

The term mycotoxin is originated from the Greek word “mykes or mukos” and means mold, and the Latin word “toxicum” which means poison. Mycotoxins are a fungi’s secondary metabolite. Fungal metabolites generally have low molecular weight and are produced by the primary metabolic processes of various fungi (Hussein & Brasel, 2001). The specific functions of mycotoxins are still considered a mystery even after 30 years of research and investigation. Aflatoxins, ochratoxins, zearelenone, trichothecenes, tremorgenic toxins, ergot alkaloids, and fumonisin are the mycotoxins of greatest agro-economic pernicious (Hussein & Brasel, 2001). These toxins are the one of the greatest worldwide life threatening and food and feed contaminants.

While all mycotoxins are of fungal derivation, however, not all toxins produced by fungi are under the term mycotoxins (Zain, 2011). In 1962, the word mycotoxin was connected to the aftermath of a significant crisis that happened in London, England, when approximately 100,000 turkeys died. The aflatoxin(s) (AFTs) were isolated and identified and linked with peanut consumption. The turkey feed was contaminated with *Aspergillus flavus* and their secondary metabolites, AFTs. This crisis promptly encouraged scientists to trace and examine the toxicity of all other ambiguous mold metabolites, which could be deadly (Bennett & Klich, 2003; Zain, 2011.)

The highly carcinogenic AFTs are produced mainly by two specific strains: *Aspergillus parasiticus* and *Aspergillus flavus*. They secrete four major types of aflatoxin: B₁, B₂, G₁, G₂, (Belc et al., 2016; Bennett & Klich, 1999; Piermarini et al., 2007). They are named blue (B) or green (G) according to their fluorescence under UV light and the differences in their chromatographic mobility in the thin-layer

chromatography (TLC). In addition, there are two other less toxic AFT metabolites designated M₁ and M₂. The aflatoxin B series, especially B₁, is the most toxic. The dominant aflatoxigenic species in crops, *Aspergillus flavus*, was divided into two different morphotypes. The two types of the *A. flavus* are the L strain and the S strain. The L strain forms a large sclerotia >400 μm in diameter. The S strain, classified as *A. parvisclerotigenus*, which basically forms several small sclerotia <400 μm (Samson et al., 2004). The L strain is excessively adaptable to AFT production, with isolates fluctuating from non-aflatoxigenic to extremely aflatoxigenic (Horn and Dorner, 1999). Generally, the S strain produces greater amounts of AFTs, unlike the L strain.

The required infectious doses of AFTs to develop harmful health effects are varied among fungi and the different infected animals or humans. Toxicological effects on humans and animals can be either acute or chronic. Acute effect is an instant onset of a harmful impact from a single high-dose exposure, whereas chronic toxicity is gradual or delay symptoms and is typically due to multiple or long-term exposures. Chronic toxicant is the primary mechanism of infection, generally associated with ingestion of low doses over long periods of time creates difficulties across the body (Hussein & Brasel, 2001; Makun, 2013). Aflatoxins cause hepatocellular carcinoma (liver cancer) especially in combination with the virus of hepatitis B in animals and humans. Regarding the mentioned hazards, the maximum tolerance levels of AFB₁ allowed is 4–20 ng/g in food and feed (Beasley et al., 1981; Hussein & Brasel, 2001; Turner et al., 2009). As is the case with any contaminating food agent, the amount of consumed contaminants and the infectious dose should always be considered.

Aflatoxins Regulation

Food and health organizations through over 100 nations throughout the world have established food-safety regulations for AFT maximum tolerable levels. These standards were to protect public health and avoid lethal crises attributed to AFT consumption (Wu & Guclu, 2012). For instance, in Kenya in 2004, 125 people died from ingesting homegrown corn, which was contaminated with AFT above-tolerance levels (Lewis et al., 2005). After exposure, AFTs are bioactivated in humans via the cytochrome P450 enzymes to start their toxic effects. Once activated, AFT can bind to and damage cellular targets such as DNA and proteins (Guengerich et al., 1998). The high lipophilic nature of this toxin facilitates its crossing of the placental barrier increasing fatal exposures. These exposures have led to immunodeficiency, altered growth factor behavior, and intestinal toxicity in children (Wild et al., 1991). Due to the highly lethal nature of AFTs, enacted, food regulations are very stringent with regard to the amount allowed in foods for human consumption. Therefore, most of the world's nations regulate the sum of the four most existent types of AFTs in food: B₁, B₂, G₁, G₂, and aflatoxin M₁ in dairy products (Van Egmond, 1989). Within the European Union (EU), aflatoxin B₁ content must be <2 ng/g. Total AFTs must not exceed 0.1 ng/g in children's products and 4–15 ng/g for other products (EU Committee). The FDA regulations stipulate that AFT concentrations must be ≤ 20 ng/g for all foods. Australia and Canada put a restriction for the total AFTs in nuts not to exceed 15 ng/g for all foods (Wu & Guclu, 2012). The same was recommended from the international limit for raw peanuts by the Codex Alimentarius Commission (CAC).

The European Scientific Committee for food (ESC) concluded that aflatoxin B₁ is genotoxic, therefore, no tolerable daily intake can be set. Similarly, aflatoxin M₁ is

regulated in at least 14 countries; the permit levels generally are within the range of 0.05–0.5 parts per billion (Coker, 2000). Although these regulatory standards are supported in the United States, the European Union (EU), and in developing countries, it is common for commodities to be contaminated with concentration levels >100 ng/g. In fact, the variations between the AFT standards in the most industrial countries are somehow quite large, which affect the global market. This leads to the urgent demand of harmonization of mycotoxin regulations all around the world (Van Egmond, 1989). In industrialized countries, the impact of food contamination with AFTs adversely affects the economy more than the public health (Wu & Guclu, 2012). The health impacts of AFT in low-income countries are more severe. Many individuals are exposed to high levels of AFTs which affect them chronically through the essential foods such as maize and peanuts. This usually leads to frequent deaths from liver cancer and aflatoxicosis. Low-income countries are suffering from lack of the resources, infrastructure services, and technology, which are important for daily food control and AFT monitoring. Aflatoxin exposures are generally highest in Asian and in sub-Saharan African nations.

Through the examination of many types of agricultural commodities, many researchers have suggested that the peanut is the most frequently contaminated with AFTs among all other crops. This has been attributed to such factors such as high moisture and nutrient content, methods of harvest, and storage circumstances, which could provide favorable conditions for the growth of contaminating molds (Wogan, 1966). Usually, aflatoxigenic fungi's best reservoir is the soil which works as a source of crops' infection such as peanut because it grows underground, with the developing pods contacting directly with the soil's living organisms. This makes peanuts one of the

most susceptible seeds to be invaded by *A. parasiticus* and *A. flavus*, especially under conditions of stress, high temperatures, and drought. Usually, damaged peanut kernels contain the highest AFT concentrations (Horn and Dorner, 1999; Lee & Krochta, 2002).

Furthermore, the peanut is considered to be one of the highest produced and consumed seeds. Peanut world production is approximately 29 million metric tons yearly. The United States is the third producer in the world. Roughly 2.4 billion pounds of peanuts are consumed yearly, one-half of it as a peanut butter; approximately 90 million jars of peanut butter are annually sold. In addition, peanuts and its products covered two-thirds of all nut snacks consumed in the U.S (Jain and Lee 2006).

Individual consumption is 6 pounds of peanuts or peanut products annually in the U.S. (Beyer et al., 2001). Seven states produce 99% of all U.S. production of peanuts. The major producing state is Georgia with 41% production, then Texas with 24% production, Alabama produces approximately 10%, followed by North Carolina 9%, Florida with 6% production, Oklahoma 5%, and finally Virginia with just 5% of the total yield (American Peanut Council, 2017). In addition, there is a noticeable increasing demand for all peanut products all around the world; for instance, peanut oil, peanut butter, salted and roasted peanuts, and peanut confections.

U.S. peanuts fall under four categories: Runner, Virginia, Valencia, and Spanish. Each of these peanuts types are distinguished by the differences in their size and flavor. However, in the early 1970s, Runner became the most commonly grown peanut in the U.S. Runner has an attractive kernel size range which is the reason for gaining wide acceptance through the U.S. The majority of Runner peanuts are used for peanut butter

production. Runner is now 80% of the total U.S. production, mainly grown in Georgia, Florida, Texas, Alabama, and Oklahoma (American Peanut Council, 2017).

AFT Detoxification Methods

AFTs are considered one of the greatest food-safety concerns. AFTs are a major economic problem for the peanut industry, which must spend more money for monitoring procedures to confirm that all products are going to the consumers free of aflatoxin (USDA). Several methods since the 1960s has been used for AFT detoxification. The Food and Agriculture Organization (FAO) stipulate that the method should:

- Inactivate or destroy, or eliminate aflatoxins.
- Not to leave nor produce toxic or carcinogenic or mutagenic residuals in the final food products.
- Increase the overall safety and avoid formation of harmful residues or leave any toxic substances.
- The new technique should not adversely affect or suppress the nutritional value and the desirable sensory and physical properties of the treated product.
- It should be applicable technically and feasible economically.
- Initially, it should be potent to kill the spores and destroy mycelium of the toxic fungi and inhibit its ability to proliferate or produce toxins under optimum conditions again (Cole et al., 1985; Piva et al., 1995; Rustom, 1997).

This review reveals some of the most effective chemical, physical, and biological approaches for AFT detoxification throughout the last four decades.

Chemical Approach

Several chemicals have shown a high potency to react with AFTs and adversely affect the AFT's harmful impact by changing their chemical structure to less carcinogenic and toxic substances. These chemicals involve bases, acids, bisulphites,

gases, and oxidizing agents. Most experiments ended in the formation of other toxic residues and caused unfavorable degradation in sensory, functional, and nutritional properties of the food. However, ozone and ammonium treatments have been used to destroy AFTs in peanut meal, cottonseed, and maize, especially for animal feed (Rustom, 1997).

Using the ozone in AFT detoxification

FDA, in 1982, stated ozone as a generally recognized as safe substance (GRAS) for bottled-water disinfection use only (FDA, 2008). It has started to be used in food processing (Kim et al., 1999; Mahapatra et al., 2005). Ozone has gained positive reputation in the food sanitization field instead of chlorine, as chlorine has led to environmental and occupational problems (EPA, 1999). Even though, this substance still needs to be tested to confirm its efficiency to be safe economically, and healthy substitution of many other chemicals.

Ozone is a gas at room temperature. Human olfaction can detect ozone's odor at levels as low as 0.00002 g/m^3 , which is 10 times below the WHO limit of recommended exposure for 1 h. The highly oxidation potency O_3 of -2.07 volts (V) makes it 1.5 and 1.3 times more lethal than hydrogen peroxide and chlorine, respectively, against many micro-pathogenic species (Foegeding, 1985; Freitas-Silva & Venâncio, 2010; Khadre et al., 2001).

Although, there are some encouraging results recorded from applications of using the ozone to inhibit the growth of some filamentous fungi or their toxins. In several studies, ozone was found to be able to destroy microorganisms and alter their metabolites toxic structure, leaving no traces of ozone in the treated crops. This fact makes the use of ozone safe in food applications.

The detoxifying effects of ozone on corn flour, flaked red paper, pistachio kernel, ground pistachios, dried figs, and corn flour were all tested using different ozone levels and treatment times. For pistachio kernel and ground pistachios, the concentrations of the used ozone were 5.0, 7.0, and 9.0 g/m³. The reduction of total aflatoxins and AFB₁ in pistachio kernels was about 24% and 23%, and the reduction of total aflatoxins and AFB₁ in ground pistachio was 5% and 93%, respectively, in 60 min (Akbas and Ozdemir, 2006).

For flaked red paper, gaseous O₃ concentrations 16, 33, and 66 g/m³ were used for 60 min, and the reduction of AFTs reached to 93% (Inan et al., 2007). For dried figs, the used concentration was 1.7 g/m³ liquid O₃ and 13.8 g/m³ gaseous O₃ for 7.5, 15, and 30 min. The reduction of aflatoxin B₁ was more effective in the gaseous treatment (Zorlugenç et al., 2008). After corn flour was treated with 15, 30, 45, and 75 mg liquid ozone for 60 min, the concentration of AFB₂, AFG₁, and AFB₁ declined from 2.42, 12.08, and 53.60 µg/kg to 0.71, 3.37, and 11.38 µg/kg, respectively. From the previous studies, it became obvious that as the concentration of the ozone and exposing time increased, the contents of AFTs decreased. Ozone either partially degrades AFTs or causes chemical modification. Investigations revealed that the degradation by chemical alteration was found just in AFG₁, and AFB₁; however, AFG₂, and AFB₂ showed a greater resistance (Cullen & Newberne, 2013).

The mechanism of ozone on the detoxification of AFB₁ and AFG₁ involves an electrophilic reaction of the C8-C9 double bond in the furan ring causing the formation of ozonized species. These compounds are then rearranged into monozonide byproducts such as ketones, aldehydes, carbon dioxide, and acids (Alexandre et al.,

2012; Lou et al., 2004). Since there is no C8-C9 double bond within the structure, AFB₂ and AFG₂ are more resistant to ozonization than AFB₁ and AFG₁ (Agriopoulou et al., 2016; Chen et al., 2014). Even though the efficiency of ozone as a chemical detoxifier is high, a greater concentration is required to kill fungi or contaminated surfaces, while low concentration of ozone and short fumigation time is generally considered necessary in order to preserve product properties like color, flavor, aroma, and vitamins (Bocci, 2010; Chen et al., 2014).

The disadvantages of using the ozone for AFT detoxification: The ozone oxidizes polyunsaturated fatty acids, sulfhydryl group, peptides', enzymes', and proteins' amino acids to smaller molecular fragments; ozone degrades the cell wall envelope of unsaturated lipids resulting in leakage of cellular contents (Das et al., 2006). Ozone treatment significantly affects the moisture content of the treated food, especially when used in high concentration (Lou et al., 2004).

Ammonia NH₃ treatment

Exposing to ammonia in solution, in the gaseous phase, or with other compounds that have the ability to release it, fulfilled optimum results in detoxification of several crops such as peanut, corn meal, and cotton. Efficiency is related to the amount of ammonia used, reaction time, levels of pressure and temperature, and being combined with formaldehyde. The mechanism of action induced by ammonia on aflatoxin AFB₁ is that the chemical structure of this toxin is irreversibly changed after the reaction with ammonia for a long time. In contrast, if the exposure time wasn't sufficiently long, the AFBs molecule structure can return to its original structure (Allameh et al., 2005; Piva et al., 1995; Weng et al., 1994). A study conducted on yellow corn naturally contaminated with 12,500 ng/g of AFB₁ and treated with ammonia hydroxide (NH₄OH)

at high temperatures, showed AFB₁ degradation greater than 99% (Weng et al., 1994). Several studies on ammonia treatment revealed that the high-temperature aqueous NH₄OH or gaseous NH₃ can eliminate AFTs successfully from food and animal feed.

It has been proposed that the resulted compound from the ammonia AFB's reaction is the less toxic than Aflatoxin D (AFD) which is a non-fluorescent phenol with molecular weight (MW) 286. Its structure doesn't include the lactone carbonylic functional group, which is considered the reason of the toxicity of AFBs. AFD₁ was biologically examined in order to determine the potential toxicity and mutagenic potency of the residual. Results showed that AFD₁ is as much as 450-fold less toxic than AFB₁.

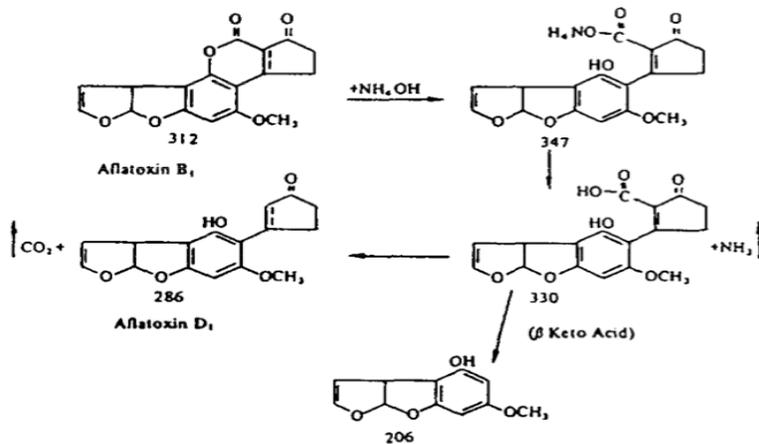


Figure 2-1. Ammonization of aflatoxin (Piva et al., 1995).

Ammonia treatment has some disadvantages, this chemical decomposes metals. In addition, ammonia usually forms unfavorable brown color in food, which cannot be avoided. The elevation in non-protein nitrogen, and in total nitrogen parallel with a significant decline in the solubility of the nitrogen, in the amino acids content such as methionine, cystine, and especially lysine, and protein efficiency ratios (Dollear et al., 1968; Piva et al., 1995).

Addition of sorbents

Materials such as silicas, aluminas, and aluminosilicates are being used to pursue specific approaches intended to reducing the AFM₁ carryover in milk, meat, and egg. These materials have the ability to decrease the AFT absorption in the animal intestine. Several studies have been conducted on different types of animals on using hydrated sodium calcium aluminosilicates (HSCAS). This material can bind AFTs and reduce the production of AFM₁. In addition, an experiment had examined bentonite as an AFT sorbent in dairy cows and the results showed a 33% decline in the AFM₁ carryover, while in vitro trials on trout feed accomplished adsorption of 70% AFB₁ in the feed (Veldman, 1992). Another in vitro test confirmed the efficiency of the MycoBond, which is a commercial product made of improved chemical phylloaluminosilicate accompanied with multiple-layer montmorillonite, and formed an inert stable complex potent to prevent the absorption of mycotoxins in the intestine (Piva et al., 1995; Winfree, 1992).

Citric acid treatment

It was hypothesized that acid treatments have the ability to alter the chemical structure of B series AFTs. The converted substance has mutagenicity 1000 times lower than the original toxin (Rustom, 1997). Ciegler & Peterson (1968) had tested the effect of citric acid on AFB₁ by adding 500 AFB₁ to 1 liter of 0.1 N citric acid. The solution was agitated at 28°C for 24–48 h. He noticed the production of a new compound which was more polar and differed in terms of fluorescent intensity. For all its physical properties, the researcher suggested that this compound could be hydroxydihydro-aflatoxin; however, this reaction is reversible. Chemical inactivation of AFB₁ and AFB₂ in maize grain, duckling feed, and extruded sorghum was tested by using 0.1 N aqueous citric

acid. Conversion was measured by the AFLATEST™ immunoaffinity column method, high-performance liquid chromatography (HPLC), and the toxicity were tested using the improved Ames test. Results revealed that treatment with aqueous citric acid achieved 96.7%, 86%, and 92% degradation in AFTs concentration in those foods, respectively. In addition, the AFT B series fluorescence intensity of the acid treated samples was far lighter than the non-treated samples as appeared in the chromatograms of the HPLC. On the other side, the results from the Ames test showed a great reduction of the mutagenic ability of the acidified maize (Ciegler & Peterson, 1968; Méndez-Albores et al., 2005). In 2010, rice was treated with 1 N aqueous citric acid to destroy AFT. The AFTs in rice samples were 97.22% degraded. However, the best degradation occurred in rice contaminated with ≥ 30 ppb (Safara et al., 2010).

Biological Approach

One of the most familiar methods for the mycotoxins quality control in foods is using microorganisms to biologically decontaminate mycotoxins. *Saccharomyces cerevisiae* and lactic acid bacteria, two possible decontaminating microorganisms, have proven that their detoxification abilities are significant amongst the other microorganisms. Yeast and lactic acid bacteria (LAB) are best known to bind variant molecules. For example, structures on the cell wall surface could bind some toxins or metal ions. In addition, the wall structure of both yeast and LAB is completely different, which results in the different mechanism of binding molecules in both cells (Juodeikiene et al., 2012). According to some studies reported, they confirm that the binding process, with AFTs, is by adhesion to the cell wall components rather than by metabolism or by binding covalently (Shetty & Jespersen, 2006).

Adsorption of mycotoxins by yeasts

In the 1980s, when there was some information regarding the usage of zearalenone (a mycotoxin) contaminated maize of ethanol production, after the fermentation process, some unusual action was discovered. The included toxins disappeared in the residual substances, which garbled the hypothesis that the toxin by some means might be bound to yeast cells. A recent study tested isolates of yeasts from several species, including *Candida krusei* and *S. cerevisiae*, for AFT binding. Toxin binding was found to be highly strain specific. Specific isolates from maize from West Africa was found to have the ability to bind with 60% of the added toxins in phosphate buffer saline (PBS). Most of the yeast strains could bind with 15% of aflatoxin B₁. Although there are numerous reports of animal feeding experiments on yeasts and their cell wall components, resulting in different levels of degradation of AFT toxicity, induced by some fungi's secondary metabolites, still there are no precise reports on a specific strain of *S. cerevisiae* that could bind AFTs under laboratory circumstances (Shetty & Jespersen, 2006).

The cell wall is a greatly dynamic structure responding rapidly to changes in the environment and stress. Regarding the physical nature and chemical composition of the *S. cerevisiae* cell wall, it is rational to think that the cell surface presents numerous sites on its surface for physical adsorption of molecules. Some studies revealed that mannan components of the *S. cerevisiae* cell wall has a role in AFT binding. The cell wall of the *S. cerevisiae* is a bi-layered structure and considers 30% of the cell total weight and its content of up to β -1, 3-glucan and β -1, 6-glucan. Mannoproteins proteins are the major protein in the cell wall. They have a covalent link to β -1, 3-glucans through β -1, 6-glucan chains. They contain an actual heterogeneous type of glycoproteins; 70 of them have

been identified. Another important piece is the phosphodiester bridges in the mannosyl side chains, which provide many negative charges at the cell surface, which could be the possible sight of mycotoxin binding.

Adsorption of mycotoxins by lactic acid bacteria (LAB)

Many scientists are supporting the idea that the best technique for AFT decontamination should be by using specific microorganisms, giving an advantage of eliminating the toxins under mild conditions, avoiding the use of harmful chemicals, and without a substantial loss in nutritive value, in addition to the provided safety to the food and feed.

Using lactic acid bacteria in fermenting food is practice for centuries. Early studies revealed that several strains of LAB are AFT biosynthesis inhibitors but not sufficient factors in AFT detoxification. When five isolates of LAB, including *Lactobacillus rhamnosus* strains GG and LC 705, *L. casei* Shirota, *L. gasseri*, and *L. acidophilus*, were screened for AFT binding (LAB cell wall components, peptidoglycan, including lipoteichoic and teichoic acid, neutral polysaccharides, and proteinaceous S layer) all played diverse functions in macromolecular binding and adhesion to AFT. Both the probiotic *L. rhamnosus* strains effectively removed aflatoxin B₁ from inoculated culture media. The destruction was through a fast process immediately eliminating around 80% of AFTs. *L. rhamnosus* strains GG and LC705 which was found effectively binding to aflatoxin B₁ better than any other AFT (EI-Nezami et al., 2002). Furthermore, similar AFB₁ binding strategy had reported from these two strains, even though they had some differences in respect to other toxins (Shetty & Jespersen, 2006).

Enzymes for detoxification

There have been numerous reports of using microbial enzymes such as AFO (aflatoxin oxidase) for AFT detoxification. One of the most known enzymes is isolated from the mushroom of *Armillariella tabescens* (Liu et al., 1998). This enzyme demonstrated high AFB₁ degradation ability and has been named aflatoxin detoxifizyme (ADTZ). A study in 2015 used AFO in a concentration as small as 0.2 mg/ml with a specific enzyme activity of 180 μ /mg revealed that this negligible amount can decline 1 μ mol of AFB₁ per minute at 30°C (Wu et al., 2015). AFO is a selective enzyme for AFB₁ or similar structures, such as sterigmatocystin (ST), versicolorin A, or any chemical that has a furan or pyran rings. The proposed site for AFO to act with AFB₁ is the bisfuran structure. The production of hydrogen peroxide is important to induce the AFO effectiveness.

Physical Approach

Fungi-contaminated seeds can be eradicated by hand selecting or using photoelectric detecting machines such as X-ray images and Computing Tomography (XCT) to detect and separate the infected kernels (Kotwaliwale et al., 2014; Pearson & Wicklow, 2006). This method is time and money consuming. However, other physical aspects are more logical and successful in terms of fungi infection elimination and AFT inactivation. These methods include: solvent extraction, adsorption, and inactivation by heat and irradiation (Rustom, 1997).

Extraction

Organic solvents such as chloroform, methanol, hexane, and acetone have been used to extract AFTs from agronomic crops.

Extraction by chemical solvents was used to eliminate AFTs from the cottonseed and oilseeds peanut. Scientists suggested that materials treated by these chemicals are only appropriate for animal feed. The typical used solvents are: 80% isopropanol, 90% diluted acetone, 95% ethanol, methanol-water, hexane-methanol, acetonitrile-water, and acetone-hexane-water. The ratio of solvent:sample is considered critical for the recovery of the AFTs (Cole & Dorner, 1994). The extraction method can eliminate all traces of AFTs from oil seed with no harmful residual formation or protein reduction in treated food. However, this technique is very expensive in large-scale applications compared with other detoxification techniques, in addition to the crucial problems related to the difficulties in the disposal of the leftover toxic extracts (Rustom, 1997).

Heat

Aflatoxins are considered heat resistance substances. They have high breakdown temperatures varying from 237°C to 306°C. AFB₁ is relatively stable under dry heating at temperatures below its thermal decomposition temperature of 267°C. Using temperature to eliminate AFTs from contaminated food and food products is a logical technique to be tested. However, heating using the regular home cooking conditions like frying or boiling at approximately 150°C have failed to remove AFB₁ and AFG₁ completely. Many variables have been connected directly to the extent of AFT destruction; the type of the food, the initial level of contamination, the heating temperature, cooking time, pH, and ionic strength of the food. However, the most crucial factor affecting the degradation of AFT was found to be the moisture content. Heat easily inactivates AFTs in contaminated foods that have high moisture content. Mann et al. (1967) have noticed that when cottonseed meal containing 30% moisture was heated to 100°C for 1 h, 74.8% of AFB₁ and AFB₂ was destroyed, while only 32.7% of

the AFTs were inactivated after heating the same meal at the same temperature when it has 6.6% moisture content. Several studies have been conducted on AFT inactivation in peanuts by high temperature, roasting or boiling in different means; direct roasting at 204°C for 20 min showed 52% reduction. The dry heating treatment at 150°C for 30 min for AFT contaminated peanuts reduced AFB₁ to 45% (Pluyer et al., 1987). AFB₁ inoculated peanuts treated with autoclave moist temperature 116°C, under pressure 0.7 bar for 30 min, and in 5% NaCl brine, recorded an outstanding reduction reaching to 80%–100% (Farah et al., 1983). Even though the direct and indirect high-heat treatments could be effective in terms of AFT detoxification in peanuts, the final quality has been questioned, given that the high temperature may degrade the nutrient's value and produce unfavorable compounds.

Irradiation

Radiation is divided into two classifications: ionizing and non-ionizing. Ionizing radiation includes gamma ray, X-ray, and ultraviolet rays. The irradiated molecules possibly change when exposed to small increase in temperature. On the contrary, non-ionizing radiation such as radio wave, microwave, infrared wave, and visible light in high intensity may increase the temperature significantly, which leads to changes in food structure with no known hazard to human health. Despite the doubts about the safety of irradiated food, it has become a technique for commercial-scale food product sterilization.

Microwave

Microwave treatment showed sufficient results in terms of AFT destruction in pre-contaminated peanuts. Reduction was dependent on the level of the power and the treatment time. For example, roasting peanuts artificially contaminated meal in a

microwave at 6 kW for 4 min eliminated 95% of the meal AFT content (Yazdanpanah et al., 2005). Likewise, microwaving decreased the AFT levels in peanut kernels at low energy at 0.7 kW for 8.5 min resulting in 48%–61% of AFB₁ reduction in the peanut kernels which were artificially contaminated. However, the same treatment resulted in only 30%–45% reduction of AFB₁ in the peanut kernels which were naturally contaminated (Pluyer et al., 1987).

Ultraviolet (UV) light

Several methods since the 1960s, were investigated for AFT destruction or elimination purposes. UV irradiation with its four regions of wavelengths: vacuum UV 100–200 nm, UV-C 200–280 nm, UV-B 280–315 nm, and UV-A 315–400 nm, was the most tested method because AFTs showed sensitivity to UV radiation. AFTs absorb UV light at 222, 265, and 362 nm, with maximum absorption at 362 nm, which leads to the production of up to 12 photo-destruction compounds which are less toxic (Krishnamurthy et al., 2008; Samarajeewa et al., 1990). AFB₁ and AFG₁ were subject to multiple photochemical changes when exposed to UV light 365 nm for 1 hour. Protein bound AFTs showed less susceptibility to photo-destruction than the free toxins (Shantha & Murthy, 1977). Standard vials of AFT with different solution concentrations of 1000 µg/kg AFB₁, 200 µg/kg AFB₂, 1000 µg/kg AFG₁, 200 µg/kg AFG₂, and 2400 µg/kg AFT were treated by UV-irradiation at 366 nm wavelength for 10 min. Degradation was by 98, 99.5, 99.8, 100, and 99.1%, respectively (Sharareh et al., 2015). Peanut samples were treated by UV 260 nm, intensity (108 J/cm²) for 45 min at room temperature and the reduction in AFT content was 87.76–96.49% (Jubeen et al., 2012). From previous studies, UVA, which is considered the highest penetration region and the lowest energy, has shown to be less effective than UVC, which has been concluded as

the most effective region in terms of detoxified AFTs (Diao et al., 2014). Another recent study also used the UV radiation intensity of 6.4 mW cm² to treat peanut oil with a thickness of <3 mm. Results verified that (UV) radiation reduced the AFB₁ in peanut oil from 51.96 to 7.23 µg kg⁻¹, 1 in 10 min and reduced by 86.08% (Diao et al., 2015). Raw milk was initially inoculated with AFM₁ and exposed to UV light for 20 min at 25°C. The AFM₁ declined by 60.7%, and the destruction mechanism was attributed to the double bond opening in AFM₁ terminal furan ring (Yousef & Marth, 1986).

In addition, artificially AFB₁ contaminated (250 ppb) dried figs had exposure to UV irradiation for 30 min. The treatment caused 45.7% reduction in the toxin level (Altug et al., 1990). Sixty minutes of UV and enzymatic exposure for AFB₁ contaminated red chili powder showed a reduction by 87.8% (Tripathi & Mishra, 2010). Another study stated that the exposure to short waves (254 nm) and long waves (362 nm) of UV for 30 min destroyed AFB₁ completely in wheat grains (Atalla et al., 2004). These studies support that UV irradiation is an effective technique in AFT termination, although the efficiency of detoxification is varied with the irradiation condition differences.

Pulsed light (PL)

Pulsed UV light is a relatively new technique modified and developed from continuous-wave UV light (CW UV), which was discovered in the 1930s. This broad-spectrum electromagnetic energy (100–1200) ranges from UV to the infrared region. Pulsed light contains approximately 54% UV, 25% visible, and 20% infrared light (Yang et al., 2011). This light is rich in UV-C (200 to 280 nm), which is often used to kill microorganisms. The difference between UV and PL is that the UV technique works continuously, while the PL emits discontinuous pulses. In addition, both have different lamp types; the most used lamps in continuous UV light techniques are low-pressure

(LP) and medium-pressure (MP) mercury-vapor lamps known as CW-UV (Diffey, 2012). The LP mercury lamp is the oldest; it discharges monochromatic wavelength at 254.7 nm, and this wavelength is close to the 260 nm, which is a germicidal effective wavelength. UV-C is usually used to preserve food and surfaces. A more developed technique is MP UV-C lamps, which release polychromatic light at wavelengths between 200 and 300 nm. The wavelength between 254 and 264 nm has the highest germicidal properties. UV light absorbed by microorganisms induces photo-chemical and photo-thermal effects. Therefore, in order to improve the absorbance of light by microorganisms, innovative lamp design is essential (Sandeep, 2001). Consequently, the PL works with xenon or krypton lamps, which can emit a number of flashes per second (Gómez-López et al., 2007). The PL energy is magnified multiple folds by accumulating in a capacitor in a fraction of a second then releasing it as short-duration discontinuous pulses using a lamp full of ionized inert gas, such as xenon. These pulses form an intensive light within a short time (nanoseconds to milliseconds with a negligible amount of additional energy consumption (Gómez-López et al., 2007; Oms-Oliu et al., 2010). This provides an amplification for the UV intensity to approximately 20,000 times more than the conventional, continuous mercury UV light, and the sunshine at sea level (Dunn et al., 1995; Krishnamurthy et al., 2007). PL is four to six times more efficient than continuous UV light in terms of pathogen inactivation (Krishnamurthy et al., 2008).

PL has not been sufficiently studied in terms of AFT illumination. Studies, such as mycotoxins' degradation in solvent (Moreau et al., 2013), food allergen reduction (Yang et al., 2010) and enzyme inactivation studies have been conducted. The PL spectrums have three main effects on materials: photo-chemical, photo-thermal, and

photo-physical (Shriver et al., 2011). For instance, Moreau et al. (2013) recorded that eight flashes of pulsed light can eliminate 72.5 ± 1.1 , 84.5 ± 1.9 , 92.7 ± 0.8 , and $98.1 \pm 0.2\%$ of deoxynivalenol, zearalenone, aflatoxin B₁, and ochratoxin in solution, respectively. Their work showed that this type of irradiation can eliminate some mycotoxins.

Although most PL detoxification methods for AFTs were effective at some level, the method could not achieve a sufficient level of AFT degradation accompanied with maintaining food quality. However, many successful experiments were conducted using PL to eliminate or destruct several types of microorganisms and allergenic compounds. Therefore, this study will continue the required investigation on the ability of the PL to destroy specific types of aflatoxin (AFB₁ and AFB₂) in inoculated peanuts, which is considered an important worldwide crop.

Justification of Study

Aflatoxins grabbed the world's attention because of the massive economic loss due to the noxious influence they have on human health and animal livestock industries in both local and global markets. The annual estimated losses from this progressive dilemma in Canada and the USA were U.S. \$5 billion (Lyn et al., 2009). In developing countries where basic foodstuffs such as maize and peanuts are susceptible to be contaminated, premature death and morbidity is associated with the consumption of AFTs. Several methods have been used to detoxify or destroy AFTs in different crops (Coker, 2000). However, AFTs are still considered an elevated risk contaminating substance because in terms of thermal methods, the AFT B series is relatively highly resistant to thermal degradation. A complete destruction is hard to achieve even at temperatures of around 250°C in dry grains (Magan & Olsen, 2004; Torres et al., 2001).

Given AFT's resistance to thermal degradation, this study will use PL as a semi-thermal AFT degradation method.

CHAPTER 3

INACTIVATION OF AFLATOXINS B₁, B₂ IN WITH-SKIN AND WITHOUT-SKIN PEANUTS BY PULSED LIGHT AND THE EFFECT OF PULSED LIGHT ON PEANUT PROPERTIES

The natural incidence of aflatoxin(s) (AFTs) in feed and food is unavoidable and unpredictable creating a unique challenge for scientists that study food safety. These toxicants are highly abundant in tropical and subtropical areas, where the weather is warmer and humid more than other places. These conditions are optimum for the growth of fungi and their products. The growth of AFT producer fungi in some grains naturally is influenced by several factors, including the type of the grain, temperature, moisture content, fungal species, existence of minerals, relative moisture of the surrounding air, and the physical condition of the kernels (Viquez et al., 1994). Peanuts as one of the most consumed commodities around the world have grabbed attention for high susceptibility to fungal growth, such as *A. flavus*, and ultimately AFT production. The AFT dilemma needs to be studied from all aspects starting from the cause to the elimination and destruction effects. *A. flavus* is the dominant AFT producer in peanuts. Broken, immature, undersized, de-shelled, discolored, and rancid peanut kernels are most susceptible to fungi contamination (Beuchat, 1987; Chiou et al., 1984; Rucker et al., 1994).

Most of the food-safety community has emphasized that farms, manufactures, and markets should take steps to prevent peanuts fungi contamination starting from pre-harvest stage. An effective treatment is urgently needed in food processing and safety fields. Multiple chemical treatments (ammonia, sodium bisulfite, hydrogen peroxide, ozone, chlorine, acids, and alkali), and physical treatments (heat, gamma irradiation, microwave radiation, visible light, and ultraviolet) have been studied to destroy AFTs

and fungal sustainability in several types of food. However, few methods have been tested on peanuts because most of these methods have major drawbacks in terms of lacking the expediency for use in solid food, limited AFT elimination, retention of some toxic residual compounds, change of some nutrients in the food, or the formation of unfavorable byproducts (Wang et al., 2016).

Many factors influence the effectiveness of any detoxification method, including the chemical persistence of the AFT, the conditions of the method, the interaction, and the matrix of the food. Practically, a good and efficient mycotoxin detoxification method should destroy, detoxify, or eliminate the AFT; treatment must not produce any toxic residual in the food, keep the high nutrient value of the food, and retain or improve the consumer acceptability of the product (Park, 2002).

Several irradiation methods such as microwave and PL have been investigated for AFT detoxification purpose in peanuts. AFB₁ contaminated peanuts were treated in a microwave for 0.7 kW for 8.5 min resulting in just reduction of about 48–61% of AFB₁ level in peanut, but the same treatment caused only 30–45% AFB₁ decline in the naturally contaminated peanut (Pluyer et al., 1987).

Pulsed light treatment is a relatively novel food processing and preservation technology. This technique uses pulses of intensive broad spectra, including 54% UV, 20% infrared, and 26% visible light (Shriver et al., 2011). During PL radiation, the inert gas inside the PL lamp, such as Xenon, is excited by high voltage, and when these Xenon molecules want to come back to the ground state, energy is released as photons and absorbed by the food particles, leading to photo-physical, photo-thermal, and photo-chemical effects on foods (Choi et al., 2010; Shriver et al., 2011).

The former three photo-effects are responsible for the assumed mechanistic effect of PL on food or any targeted molecules (Krishnamurthy et al., 2007). The technique of PL was originally accepted as a non-thermal technology used for microorganism elimination. Nevertheless, recent study has clarified that PL may also have a photo-thermal effect in addition to its non-thermal nature. The prolonged treatment (e.g., minutes) has led to temperature elevation and moisture evaporation inside the food matrix (Yang et al., 2012). Although PL is known as a non-thermal technique, with a relatively long time process, about 30 s or more depending on a PL strobe distance from food, heat will be generated (Faidhalla, 2013).

In addition, this technology showed promising results in terms of AFT reduction. Moreau et al. (2013) recorded that eight flashes of PL can eliminate 84.5 ± 1.9 , 72.5 ± 1.1 , 92.7 ± 0.8 , and $98.1 \pm 0.2\%$ of deoxynivalenol, zearalenone, ochratoxin, and aflatoxin B₁ in solvents, respectively. Their work showed that one or several flashes of pulsed light on zearalenone and deoxynivalenol ended with a negligible decline in the toxicity of the mycotoxins. However, the same treatment for AFB₁ showed a complete elimination of the mutagenic ability of this AFT. Therefore, the presented study was conducted to investigate the effect of PL on the aflatoxin B₁, and B₂ degradation in with-skin and without-skin peanuts.

Materials and Methods

Preparation of the Samples

Sample inoculation

Raw with skin (with testa) and without-skin (w/o testa) peanuts were purchased from a domestic market. Peanuts were autoclaved for 15 min at 115°C to eliminate any pre-contaminated fungi or AFTs. Peanuts were inoculated with *A. flavus*, which was

purchased from the American Type Culture Collection (ATCC) (Manassas, VA, US). This specific strain anamorph (ATCC® 16875™) has the highest ability of producing AFB₁, AFB₂ together, among all other strains (Wei and Jong, 1986). Fungus was proliferated on PDA media (potato starch 4.0%, dextrose 20%, 2% agar), which was distributed by a hockey glass stick for 1 min, and incubated in a lab incubator for 5–7 d (Doyle et al., 1982; Nam et al., 2009). Inoculum was incubated at 30 °C. Fungi spores were harvested from the Petri dished with a spatula, filtered using cheesecloth, and a suspension of a sterile-water spore, roughly 100,000 conidia per milliliter was prepared. The conidia were counted using a hemocytometer, 500 g of peanuts was placed in Erlenmeyer flasks (1000 ml), and 50 mL of distilled water was added and autoclaved at 121°C for 15 min and allowed to stand overnight. To minimize the moisture loss from the grain, flasks were sealed with thin polyethylene film. The film was punctured by making a few holes using a pin to avoid carbon dioxide production from respiration, which could accumulate in the head space of the flask. After the inoculation process with *A. flavus* conidia, flasks were inoculated with 25 mL of the spore suspension, incubated at 30°C in dark/night of 12/12 h for 21 d. Flasks were shaken daily for moisture and fungi distribution. After this long period of incubation, flasks were boiled in water for 1 h to stop AFT production. Moisture was optimized to 16% as in previous studies (Méndez-Albores et al., 2005; Wei and Jong, 1986). Hemocytometer calculation used Equation 3-1, 3-2, 3-3.

$$\% \text{ of viable cells} = (\text{Number of viable cells} / \text{total \# of cells}) \times 100 \quad (3-1)$$

$$\text{Dilution factor} = \text{Final volume} / \text{volume of cells} \quad (3-2)$$

$$\text{Concentration (viable cells/ml)} = \text{Average number [number of cells/number of squares]} \times \text{dilution factor} \times 10^4 \quad (3-3)$$

Moisture optimization

A. flavus's production of AFT is influenced by the competition of natural microflora on peanut pods. Factors such as moisture content are crucial for AFT accumulation in peanut kernels and the development of *A. flavus* over other microorganisms (Chiou et al., 1984). The minimum moisture content of peanuts for *A. flavus* growth is 8–10% at a relative humidity of approximately 82%. The optimum moisture content for AFT production on peanuts is 15–35% (Doyle et al., 1982). Thus, moisture was controlled and adjusted to 16%. The peanuts were kept in sealed plastic bags and stored in -18°C freezer for treatment and further analysis.

Peanut kernel moisture content was determined in triplicate, using the Association of Official Analytical Chemists (AOAC) number 925.40 method (AOAC, 1990). Using a mechanical convection oven (Precision Scientific, Winchester, VA), 5 g samples of peanuts were taken directly from the inoculum. Samples were ground to a fine powder to accelerate the moisture loss inside the oven. The following modification was required because of the high moisture content of the samples. After weighing the samples in ceramic crucible, peanuts inside the crucibles were put in the oven at 103°C for 12 h. The samples were then cooled in a desiccator and weighed. Equation 3-4 was used to calculate the moisture content:

$$\% \text{ moisture (wt/wt)} = \frac{\text{wt of wet sample} - \text{wt of dry sample}}{\text{wt of wet sample}} \times 100 \quad (3-4)$$

Uniformity test

This test was conducted in triplicate to confirm the uniformity of the peanut inoculum. *A. flavus* inoculated peanuts with and without skin samples 5, 10, and 15 g, in triplicate were used. AFTs were extracted from all samples using the same extraction

method using 70% aqua-methanol. Then a competitive ELISA test was used to determine the differences in AFT concentration in all samples. Data were analyzed by Analysis of Variance (ANOVA) using SPSS at $\alpha=0.05$, and to evaluate the significance of the differences between samples.

Pulsed UV light treatment

Pulsed light treatment was conducted using a Xenon PL applicator of Model # LHS40 LMP HSG from Xenon Corp (Wilmington, MA). This system consists of an RC-747 power/control module, a treatment chamber that contains two xenon lamps (mercury free) and two blowers (air-cooling system) at the top of the lamp housing chamber, and one treatment chamber with a conveyor belt. The xenon lamp used has an electrical efficiency of 10%–30% and UV intensity of 30.000 w/cm (Koutchma, 2009). The PL system generates a broadband spectrum between 100 and 1100 nm. Approximately 20%, 26%, and 54% from the energy are infrared, visible light, and ultraviolet regions, respectively. This system provides high-intensity PL at a pulse rate of three pulses per second and a pulse width of 360 μ s, as per the manufacturer's specification. Treatment delivers 1.27 J per centimeter squared (J/cm^2) for an input of 3800 V at 1.9 cm below the central axis of the quartz window of the PL xenon lamp (Wang et al., 2016).

The treatments were conducted in triplicate. Each time, 5 g samples of with-skin and without-skin raw runner type peanuts were arranged in small aluminum dishes (7.2 cm diameter, and 1 cm high) obtained from Fisher Scientific, Inc. (Allentown, PA). A combination of diverse illumination durations and distances were tested to explore the effective conditions for PL treatment of whole peanuts. The samples were treated by the PL for several duration times 30, 60, 90, 120, 180, 210, 240, and 300 s in three different

distances 5, 7, and 10 cm from the PL strobe. The treatment times were selected based upon the degree of visual roasting that occurred and AFTs reduction for the peanut samples, ranging from lightly roasted to burnt, during preliminary tests.

Aflatoxin extraction

After the PL treatment, 5 g of peanuts were well-milled in a grinder (Model LXC-150, 50/60Hz, 180W, Keunex, Korea). Then the peanuts were blended (31BL91, Waring, Dynamics Corporation of America, Hartford, CT) with 25 mL of 70% methanol (aqueous) for 2 min to extract AFTs. Then the extract was transferred to conical flasks and was shaken (MTS 2/4 D S1, IKA, Wilmington, NC) for 30 min at 300 rpm. The extract was allowed to settle, then filtered through a Whatman Number 1 filter paper and moved to autoclaved containers and stored in a freezer (-18°C) until analysis for AFB₁, AFB₂ content. Extractions after storage were shaken before ELISA analysis by using an orbital shaker (Heidolph, Schwabach, Germany) for 3 min similar to the method of Zheng et al. (2005) and Nyirahakizimana et al. (2013).

Competitive ELISA

An AgraQuant® ELISA Aflatoxin Kit (Romer Labs, Getzersdorf, Austria) was used to determine the concentration of AFB₁ and AFB₂. All procedures for ELISA analysis were performed according to manufacturer specifications. The kit is able to quantitate AFTs in the range of 4-40 ppb.

High performance liquid chromatography mass spectrometry analysis (HPLC/MS/MS)

Chromatographic analyses were performed on samples that had the highest reduction percentage to confirm the ELISA results by using HPLC/MS/MS on an Agilent (Palo Alto, CA) 1100 series. A thermo Scientific Hyper Sil Gold aQ (2.1 x 150 mm; 3

μm ; + guard column) was used for compound separation. Elution was performed using mobile phase A (H_2O , 0.2% acetic acid) and mobile phase B (methanol, 0.2% acetic acid). The flow rate was 0.2 mL/min, and the linear gradient used was: 0 min 90% A; 13 min 50% B; 45–55 min 100% A, 65–75 min 90% B. Electrospray mass spectrometry was performed (ESI-MS/MS) of the $[\text{M}+\text{H}]^+$ ions of the AFB₁ and AFB₂. LCQ DECA quadrupole ion trap mass spectrometer was operated in the positive electrospray ionization (ESI) mode operating with XCALIBUR 2.0.7.SP₁ (Thermo Fisher Scientific; San Jose, CA). The spray voltage was 4.0 kV; heated capillary voltage was +32 V; tube lens offset was -4 V. Nitrogen was used as the spray gas. Source and desolvation temperatures were set at 250°C. The ESI Duration was 60 min, and the number of scan events was five. A series of standards of AFB₁ and AFB₂ were also analyzed to create an external calibration curve. Standards were Sigma products: Aflatoxin B₁ (Sigma A6636-1 mg; Lot # 025M4092V); Aflatoxin B₂ (Sigma A9887-1 mg; Lot # 016M4012V).

Temperature Measurements

Infrared thermometer

An Omega OS423-LS (Omega Engineering, Inc, Stanford, CT) infrared thermometer was used for temperature measurements of treated peanuts before and right after PL treatment at the exact moment when the conveyor brought the samples out of the chamber.

In situ temperature measurements

Temperature was measured instantaneous by inserting tiny thermal sensors into three locations of the peanut kernel. Each sensor was inserted in distinctive locations (top, middle, and bottom) of the three different peanut kernels (Figure 3-2-A). The kernels were attached to the aluminum plate, using a lab-made flour glue, to avoid any

movement during the PL treatment. Samples were treated in-situ for 120 and 240 s at two distances 5 cm and 7 cm (Figure 3-2-C). Temperature was measured by a K-type thermocouple (Omegaette HH306, Omega Engineering Inc., Stamford, CT) using a Pico® 8-channel thermocouple datalogging interface (PC-08) attached to a computer running Pico software (Figure 3-2-B). Data was recorded at 1 s intervals.

Chemical Analysis

Peroxide value

Peroxides are the initial products of lipid oxidation (number of millimoles of peroxide in 1 kg of oil). The effect of PL on lipid oxidation of peanuts was investigated by comparing the peroxide value (PV) of the raw peanuts (control) with 240 s shaking treatment, 300 s shaking treatment, and 240 s in-plate treatment (the highest PL treatments). The peanut seeds were milled with a coffee grinder (LXC-15-AC220-240V, 180 W) and dried to final weight in a thermostatically controlled oven at 105°C. An oil extraction was carried out using a Soxhlet apparatus following the Soxhlet extraction official method of AOAC (1995). A 250 mL round bottom flask was washed and dried in an oven at 103°C for 25 min and left to cool at room temperature before it was weighed. Five grams of the dried sample were weighed into an extraction thimble (Advantec N08425X100MM) made of cellulose. This thimble was inserted into the extraction column with the condenser connected. Two hundred milliliter of the extracting solvent (chloroform, boiling point 60°C) was poured into the 250 mL flask and fixed to the condenser under the extraction unit. The flask was then heated by electro-thermal heater at 60°C for at least 8 h (Eshun et al., 2013). Extracting solvent was evaporated leaving the concentrated peanut oil sample for analysis.

Peroxide values were measured using the Official American Oil Chemists' Society (Hortwitz, 2002) method 965.33 for peroxide value of oils and fats. A 2.0 mL amount of peanut oil was placed in a 250 mL flask, and 20 mL of the appropriate solvent mixture (glacial acetic acid:chloroform 3:2) was transferred into a flask and gently shook. Saturated potassium iodide solution 1.0 mL was added and stirred at a slow speed for 1 min.

Then 100 mL distilled water, and 1 mL of starch solution was added to the solution, mixed with high speed stirring, and immediately titrated with 0.01 N or 0.1 N sodium thiosulfate. Solution was turned from a purple to a slight yellow or colorless, and this was considered the endpoint (these colors may be affected by the initial color of the tested oil). Blank determination was carried out under the same conditions. An expired sample of commercial peanut oil (2013) was examined for comparison. Peroxide value results were reported as mill equivalents peroxide per kilogram oil using Equation 3-5 (Eshun et al., 2013; Silva 2010).

$$PV \text{ [meq/kg]} = ((V_1 - V_2) \times C \times 1000) / m \quad (3-5)$$

Where: V_1 = volume of sodium thiosulfate for titrate the oil, V_2 = volume of sodium thiosulfate for titrate a blank. C = molar concentration of sodium thiosulfate, m = weight of samples in grams.

Free fatty acids and acid value

Free Fatty Acid (FFA) is a good indicator of oil quality because it measures the concentration of fatty acids that are released from triacylglycerols due to hydrolysis, lipase action, or even oxidation. FFA were measured according to the official method Ca 5a-40 of American Oil Chemists' Society (AOCS, 2009). Oil was extracted from

untreated (control) and PL treated with-skin and without-skin peanuts using Soxhlet apparatuses. Five grams of peanut oil were weighed into a 125-mL Erlenmeyer flask. In the fume hood, 50 mL of ethanol was added to dissolve the oil. Blanks which contain just 50 mL of ethanol were prepared for the same purpose. Five drops of phenolphthalein indicator were added to all samples. Then a titration to the endpoint (faint pink) with a standard solution of NaOH was controlled, and the volume titrated for blank and samples all were carried out for the final calculation using Equation 3-6 for FFA determination.

$$\% \text{ FFA (as oleic acid)} = \frac{(V - V_{\text{blank}}) \times M \times F}{SW} \quad (3-6)$$

Where: V = mL of NaOH required for sample, blank = mL of NaOH for the blank, M = Molarity of the NaOH in mol/L, F = molar mass of the oleic acid (28.2 g/mole), and SW = Sample weight (lipid) in grams.

In addition, acid value for untreated peanuts and treated peanuts was determined. It is defined as the number of milligrams of KOH needed to neutralize 1 g of sample. To convert percentage-free fatty acids (as oleic) to acid value, the free acid percentages were multiplied by 1.99.

Results and Discussion

Uniformity Test

The uniformity test results showed that there were no significant differences among all 5 g, 10 g, and 15 g of the inoculated peanuts regarding AFT concentration (Table 3-1). Figure 3-1 shows the difference in fungi growth distribution of the inoculum after 5 d of inoculation at the same conditions. These results were very helpful in terms of avoiding the misinterpretation of the results. As Food and Agriculture Organization of United Nation FAO and the World Health Organization and WHO indicated in the codex

alimentary of general standard for contaminants and toxins in food and feed (Codex Standard 193-1995) that AFT distribution is usually highly non-homogeneous in peanut samples, which were laboratory inoculated. Therefore, samples were prepared and homogenized extremely carefully.

Moisture Content

Moisture distribution is one of many factors affecting AFT distribution in any crop. Previous study results indicated that the elevation in moisture content enhanced nut quality and PL efficiency (Nkama & Muller, 1988; Özdemir and Devres, 1999). In this study, AFT reduction achieved in peanuts with moisture content of 16% was much higher than the peanuts with moisture content of 10% (Table 3-2) by a difference of about 30.7%.

Nkama & Muller (1988) tested several primary moisture contents in milled rice containing 1100 µg AFTs/kg rice, exposed UV light with high intensity of about 64 mW/cm² and at 36°C. Results clarified that increasing the moisture to 24% led to AFT content to decline to 351 µg/kg, and 14.1% moisture resulted in reduction to 413 µg/kg, which agreed with the former hypothesis. In the present study, moisture content was optimized to 16%. For future research, higher moisture levels, their effect on PL efficiency, and the correlation between moisture levels, temperature, and treatment times should be investigated from all aspects, including the sensory quality. Since this study's results showed the significant effect of temperature elevation and exposing time on AFT reduction percentages, which agreed with Demir (2004) who emphasized that both temperature and time are main parameters in any peanut application.

A study conducted by Kita et al. (2007) indicated that decline in moisture can directly decrease the mechanical characteristics such as hardness of roasted peanuts. Zhao (2013) has pointed out that traditional roasting process for dry peanuts gave a moisture loss of about 3% with consistent heating at 131–165°C for 15 min. However, PL-treated peanuts at 10 cm distance showed a moisture loss around 1.4%-5.6% for 5–9.5 min PL exposure and samples at 7 cm distance from the lamp showed a moisture loss of about 2.0%–8.8% for 5–7 min PL treatment. The moisture loss during the PL treatment was likely due to the higher instantaneous temperatures generated inside the peanut kernels (Zhao, 2013). Therefore, elevating the moisture content before the PL treatment was mandatory to enhance the photo-thermal and photo-chemical effect in addition to keeping the sensory quality of the roast or semi-roast peanuts. Furthermore, the moisture-heat sensitivity of AFB₁ was confirmed in foods treated with irradiation. It has been proposed that water molecules help in opening the lactone ring in AFB₁ to form a carboxylic acid in the terminal part of the ring.

Temperature Measurements

The present experiment's measurements indicated that temperature rose parallel with exposure time (Table 3-1, 3-2) in both with-skin and without-skin samples and opposite to increasing distance. The highest temperature recorded by the infrared thermometer was 181°C for without-skin and 150°C with-skin peanuts treated for 300 s at 5 cm from the strobe. The relatively high fat content (~ 50%) likely functioned as an efficient heat transfer medium leading to a rapid temperature rise in the peanuts. Furthermore, according to the infrared digital thermometer's temperature measurements of peanut samples, with-skin samples recorded lower temperature compared with without-skin samples, probably due to the skin which may work as a barrier, especially

for light reflection. The in-situ temperatures (Figure 3-5 A, B, C, D; Figure 3-6 A, B, C, D) were recorded using sensors, which were connected to a computer that has a special software to measure the temperature every second in-situ. Measurements showed that the homogeneity of temperature distribution was the best at 7 cm. The recorded temperature from all three sensors (Figure 3-2 A, B, C) were close at the 7 cm distance.

ELISA Results

According to ELISA AFB₁ and AFB₂ concentration inside PL treated peanuts and compared with a control of non-treated samples. Results were calculated using equations brought on from running a series of AFB₁, AFB₂ mix diluted standards with a calibration curve $R^2 = 0.99$. The AFB₁, and AFB₂ in solvent were treated with PL for several periods at 7 cm. High reduction percentages were achieved in 240 s at 7 cm PL treatment (Figure 3-4). The highest reduction of AFB₁, AFB₂ in peanuts achieved in this study was around $95.3\% \pm 3.47\%$ for without-skin peanuts treated for 300 s at 5 cm (Table 3-7). However, the surface was burned and the dark color wasn't distributed equally. For with-skin, the AFT reduction reached to $82.0\% \pm 16.1\%$ (Table 3-4) when treated for 240 s at 7 cm. This specific treatment was chosen regarding keeping the peanuts quality in addition to the significant AFT reduction comparing with other treatments of shorter time and closer or further distances from the strobe.

Comparably, a UV light treatment using 43 mW/cm^2 for 24 h reduced aflatoxin B₁ in milled rice from 1100 ppb to 135 ppb, which is 88% AFT reduction, while a higher intensity of 64 mW/cm^2 produced the same reduction of AFTs after 12 h of treatment (Nkama and Muller, 1988). Similar results for Wang et al. (2016) revealed that PL treatment with intensity of about $0.52 \text{ J/cm}^2/\text{pulse}$ for different times. He found out that

at 9 cm distance to the lamp, PL treatment for 80 s declined AFB₁ and AFB₂ in rough rice by 75.0% and 39.2%. Furthermore, a treatment of 15 s reduced AFB₁ and AFB₂ in rice bran by 90.3% and 86.7%, respectively (Wang et al., 2016).

Reduction of AFTs had a strong positive correlation coefficient (Table 3-5, 3-8) with the increasing of exposure time (0.79, 0.91) and strong positive correlation coefficient with the temperature elevation (0.87 and 0.95) for with-skin and without-skin samples, respectively. However, it had low negative correlation with the increasing in distances, (-0.4 and -0.31) for with-skin and without-skin samples, respectively. In addition, according to these results, the effect of the elevating in temperature on the reduction percentage was the highest attributed factor to AFT reduction percentage.

HPLC-MS/MS Analysis

Table 3-3 shows that the LC-MS/MS results agreed with the ELISA results. The reduction of pure AFB₁, AFB₂ in solvent reached 100% and 97.9%, respectively, when treated with PL for 180 s at 7 cm. Table 3-6 showed AFB₁, AFB₂ reduction in AFT concentrations inside peanuts' kernels after PL treatment, which reached to 90.3 and 95.2, respectively, for without-skin peanuts treated for 240 s at 7 cm, and reduction for AFB₁, AFB₂ reached to 87.2 and 84.7 for with-skin peanuts treated the same way. Results of AFB₁, AFB₂ mixture treatment led to a complete reduction for AFB₁, and semi-complete reduction for AFB₂ (Table 3-3).

Aflatoxin B₁ and B₂ exhibited a good ESI ionization efficiency in the positive ion mode for molecular ion m/z 313 (fragments m/z 270.3, 285.2, and 298) for AFB₁ at RT = 22.67 min. While for AFB₂ the molecular ion was m/z 315 (fragments m/z 259.2, 287.2, and 297.1) and eluted at RT = 21.79 min. MS/MS fragmentation pathway of AFBs

revealed that ions were formed by loss of carbon monoxide, oxygen, hydrogen, and methyl group (Iram et al., 2016).

LC-MS/MS chromatograms (Figure 3-8) showed the obvious decrease in AFB₁. AFB₂ never showed a complete reduction even though the initial concentration was lower than the AFB₁. These results agreed with the previous work of Basaran (2009), which recorded that the UV light of 254 nm did not degrade AFG₂ and AFB₂ but significantly reduced AFG₁ and AFB₁. AFTs have different sensitivity to UV light. Thus, to improve the UV detoxification efficiency, the type of contaminating AFTs should first be identified, and then specific UV wavelength where AFTs have the maximum absorption should be chosen before irradiation. In this study, obviously the wave spectrum used 220–1200 had a better effect on AFB₁ than on AFB₂. In general, results showed that after 240 s PL treatment for AFB₁, AFB₂; AFB₁ could not be detected and AFB₂ was barely discernible.

Factorial statistical analysis two-way ANOVA was conducted to determine the significant effects of the differences in times and distances during PL treatment on the AFT reduction percentages, and on temperature elevation (Table 3-4, 3-7). The treatments were significantly different at $\alpha = 0.05$ confidence interval. Fisher Least Significant Differences test (LSD) was conducted to determine the mean differences for both with-skin and without-skin samples.

For with-skin samples (Table 3-4), at 5 cm distance to the lamp, significant differences in the reduction percentage 64.6% was observed at the 240 s exposing time when temperature reached 136°C. At 7 cm, significant reduction differences among treatments at 240 s were recorded 62.4% at temperature 105°C. At 10 cm distance to

the lamp, there were no significant differences among treatments in terms of reduction percentage.

For without-skin samples (Table 3-7) at 5 cm distance to the lamp, significant differences started at 90 s exposing time when temperature reached 90.2°C. At 7 cm, the significant reduction 44.6% was achieved at 120 s when temperature reached 86.2°C. At 10 cm distance to the lamp, the significant reduction 45.2% was at 210 s when the temperature reached 130°C.

The LSD analysis showed increasing in the LSD between means equivalent to the increasing on distances. The same temperature with different distance resulted in different reduction levels. The effect of temperature was greater when the distances were closer. These results led towards the conclusion that the elevation in temperature is not the main factor of AFT reduction.

Aflatoxins particularly are stable against irradiation when in a solid state (Aibara & Yamagishi, 1970). Therefore, one of the most crucial conclusions from this study is that increase in peanut moisture content enhanced AFT degradation. Lower moisture content showed lower reduction. The possible interpretation of this is that UV absorption was increased at higher moisture content since water is highly UV absorbent. For opaque foods such as peanuts, the turbidity of the color reduces the penetration capability of the UV light which likely occurred for the peanut samples (Guerrero-Beltran & Barbosa-Canovas, 2004). When the matrix is transparent, it can penetrate perfectly, permitting a final decontamination of samples as was achieved when AFB₁ and AFB₂ in solvent was treated by PL (Feuilloley et al., 2006). However, in general, the effect of PL penetrates 2 mm into the samples (Wallen & Fraenkel, 2001).

Peroxide Value and Free Fatty Acids

Lipid oxidation occurs in three-stages: initiation, propagation, and termination. This dynamic process is controlled by several factors including temperature, light, oxygen, and storage time. The levels of peroxides change over time. At the beginning of shelf life, the peroxide value (PV) is low. However, during the food's life, the PV increases to the top point and then declines down again. Peroxide value (PV) and free fatty acid tests are both considered shelf-life indicators for peanuts

In this experiment, the PV was iodometrically determined and expressed as meq/kg. Results (Table 3-9) showed that after 240 s, 7 cm treatment, PV was 1.03 for without-skin and 0.89 for with-skin samples. The free fatty acids were 0.56% for with-skin and 0.55% for without-skin samples. The acid value was 1.08 for with-skin and 0.88 for without-skin samples. The acid value was alkalimetrically determined and expressed in mg KOH/g. Comparably, Pokorny et al. (2003) found that Virginia raw peanuts extracted oil contains free fatty acids around 0.72%, the acid value was 1.48, and the PV was 1.46. Thus, the peroxide values of PL treated peanuts were fully acceptable according to the USDA Foreign Agricultural Service (2004) regulations for quality indexes of peanut oil products: PV mmol/kg \leq 7.5 (1 meq/kg = 0.5 mmol/kg) and acid value (KOH) \leq 3.0 mg/g are within specification. Peanuts are considered rancid when the PV > 30 meq/kg according to the Chemistry and Technology of Oils and Fats (Chakrabarty, 2003). Recently, several studies have determined the PV of freshly roasted peanuts to be found between 1.0 and 10.0 meq peroxides O₂/kg oil and the acid value was 0.6–0.99 mg KOH/G oil (Akhtar et al., 2014; Özcan, 2003). According to PP12 USDA commodity requirements (2010), peroxide value shouldn't exceed 10 meq/kg for roasted peanuts.

Table 3-1. AFT uniformity test results. The concentration of AFTs for 5, 10, and 15 g *A. flavus* inoculated peanuts.

Peanut samples (g)	With skin (ppb)	w/o skin (ppb)
5	75.7 ± 8.13 a	69.7 ± 11.9 a
10	75.8 ± 4.86 a	74.6 ± 7.24 a
15	78.8 ± 4.32 a	77.0 ± 6.05 a

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD system for mean differences analysis.

Table 3-2. AFT concentration for peanuts with different moisture content after PL treatment for 300 s at 5 cm.

Peanut samples (g)	Moisture content %	AFT R %
1	4 ± 2.00	12.6 ± 2.13 a
2	10 ± 1.13	64.7 ± 11.9 b
3	16 ± 2.32	95.3 ± 3.47 c

R% = Reduction percentage. Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD system for mean differences analysis.

Table 3-3. LC-MS/MS analysis of PL effect on diluted mix AFB₁ and AFB₂ 100 ppb.

Samples	AFB ₁ R%	AFB ₂ R%
Non-treated AFB ₁ , AFB ₂ in solvent (control)	0 a	0 a
PL 180 s treated AFB ₁ , AFB ₂ in solvent	100b	97.9b
PL 240 s treated AFB ₁ , AFB ₂ in solvent	100b	97b

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis

Table 3-4. AFT reduction percentage in with-skin PL (in-plate) treated peanut samples using different distances and different time.

Time(S)	5 cm-(°C)	5 cm-R %	7 cm-(°C)	7 cm-R%	10 cm-(°C)	10 cm-R%
0	23 ± 2.35	0a	23 ± 2.35	0a	23 ± 2.35	0 a
30	58.5 ± 2.43	0a	55.6 ± 0.89	0 a	53.1 ± 2.82	0 a
60	62.8 ± 4.26	26.9 ± 4.2a,b	60.3 ± 0.25	20.9 ± 6.32a,b	59.1 ± 1.5	1.20 ± 1.40a
90	83.0 ± 1.64	33.9 ± 4a,b	67.2 ± 1.83	23.1 ± 8.42a,b	70.2 ± 4.10	3.63 ± 4.62a
120	105 ± 8.65	39.3 ± 4.9a,b	81.9 ± 7.21	34.5 ± 5.97a,b	71.1 ± 13.8	4.16 ± 0.00a
180	123 ± 4.85	40.1 ± 7a,b	96.8 ± 2.62	39.3 ± 7.05a,b	74.8 ± 15.4	4.39 ± 3.15a
210	130 ± 12.2	43.4 ± 17a,b	103 ± 3.25	40.3 ± 9.5ba,b	92.0 ± 8.40	15.1 ± 5.05a
240	136 ± 1.11	64.6 ± 19.4b	105 ± 7.35	62.4 ± 15.0b	98.3 ± 4.29	25.1 ± 15.0 a
300	150 ± 17.9	82.0 ± 16.1b,c	114 ± 11.2	64.8 ± 14.5 b	103 ± 5.57	32.8 ± 16.2a

* Mean ± standard deviation (n = 3), R% = Reduction percentage. Control was 128.993 ± 36.540 ppb.

Table 3-5. Correlation coefficient for in with-skin PL (in-plate) treated peanut samples using different distances and different time.

	Time (s)	Distance	% R	°C
Time (s)	1			
Distance	3.2E-17	1		
% R	0.79	-0.49	1	
°C	0.88	-0.30	0.87	1

R% = Reduction percentage.

Table 3-6. LC-MS/MS analysis for 5 g peanuts treated in-plate with PL for 240 s, and 7 cm distance from the strobe.

Treatments	AFB ₁ R%	AFB ₂ R%
With-skin	87.2a	84.7a
W/O-skin	90.3a	95.2a
Control	0	0

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis.

Table 3-7. AFT reduction percentage in w/o skin PL (in-plate) treated peanut samples using different distances and different time.

Time(S)	5 cm-(°C)	5 cm-R %	7 cm-(°C)	7 cm-R%	10 cm-(°C)	10 cm-R %
0	22.1 ± 0.67	0 a	22.2 ± 2.27	0 a	22.2 ± 1.45	0 a
30	58.7 ± 6.88	0 a	50.6 ± 0.29	0 a	45.1 ± 14.9	0 a
60	69.1 ± 4.41	32.6 ± 2.40 a	65.2 ± 0.98	23.5 ± 0.46 a,b	53.2 ± 3.69	13.0 ± 1.16 a,b
90	90.2 ± 5.03	36.2 ± 2.65 b	79.3 ± 2.26	28.8 ± 1.10 a,b	57.7 ± 1.20	3.00 ± 4.78 a,b
120	110 ± 6.47	48.6 ± 0.46 b,c	86.2 ± 0.73	44.6 ± 6.09 b	77.0 ± 0.81	30.4 ± 1.60 a,b
180	128 ± 3.97	68.6 ± 5.65 b,c	99.4 ± 0.82	60.2 ± 16.9 b,c	79.3 ± 1.10	41.3 ± 0.31 a,b
210	131 ± 3.66	76.3 ± 3.30 c	104 ± 2.56	70.3 ± 4.13 b,c	82.0 ± 2.34	45.2 ± 0.67 b
240	148 ± 3.02	86.9 ± 1.99 c,d	137 ± 0.01	77.9 ± 2.95 b,c	120 ± 1.59	50.1 ± 4.53 b
300	180 ± 12.9	95.3 ± 3.47 c,d	164 ± 2.54	80.1 ± 1.49 c	130 ± 1.61	55.1 ± 1.19 b

* Mean ± standard deviation (n = 3), R% = Reduction percentage. Control was 171.87 ± 22.2 ppb.

Table 3-8. Correlation coefficient of the reduction percentage with all other parameters in without-skin PL (in-plate) treated peanuts with the increasing in temperature.

	Time	Distance	%R	°C
Time (s)	1			
Distance	3.2E-17	1		
%R	0.91	-0.31	1	
°C	0.92	-0.29	0.95	1

R% = Reduction percentage.

Table 3-9. Peroxide value, free fatty acids, and total acid number for peanuts oil after PL treatments of peanuts.

Treatment	FFA %	Total Acid Mg KOH/g	PV
Control w/o skin	0.58 ± 0.15cb	0.93a	2.13 ± 0.25c
Control with skin	0.65 ± 0.03b	0.90a	1.32 ± 0.02c
240 s / 7 cm w/o skin	0.55 ± 0.09 b	0.88a	1.03 ± 0.10c
240 s / 7 cm with skin	0.56 ± 0.21 b	1.08a	0.89 ± 0.06c

* Mean ± standard deviation (n = 3). Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis.

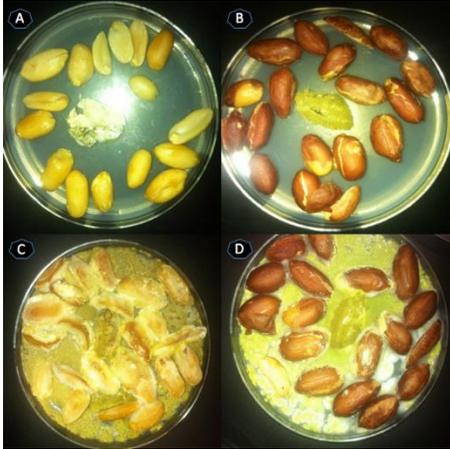
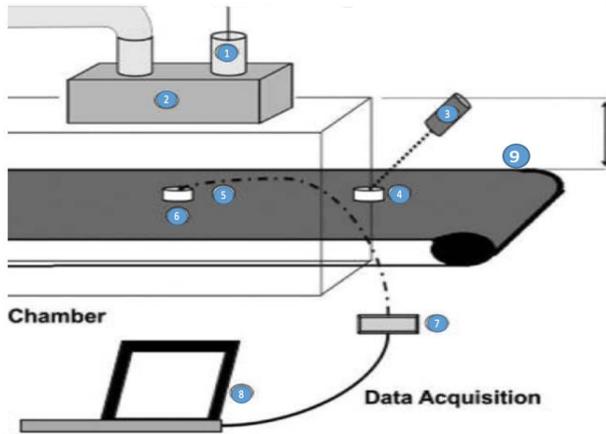


Figure 3-1. Peanuts inoculation. A) Without-skin peanuts, B) With-skin peanuts, C) *A. flavus* 5 day inoculated without-skin peanuts, D) *A. flavus* 5 day inoculated with-skin peanuts (Photo courtesy of author).



A

B



C

- 1- Blower air inlet
- 2- PUV lamp house
- 3- Infrared thermometer
- 4- Sample after treatment
- 5- T-type thermocouple (thermo-sensor)
- 6- Aluminum plate contains sample
- 7- Data logger
- 8- Computer
- 9- Distance from strobe

Figure 3-2. Temperature measurements. A) Sample's container depicting the locations of the thermo-sensors inside the peanut kernels, B) K-type thermocouple and laptop running Pico software, C) Schematic representation of PUV treatment and temperature data acquisition (Photo courtesy of author).



Figure 3-3. Xenon pulsed light machine. Model# LHS40 LMP HSG from Xenon Corp (Wilmington, MA) (Photo courtesy of author).

PL treatment for AFBs in solvent

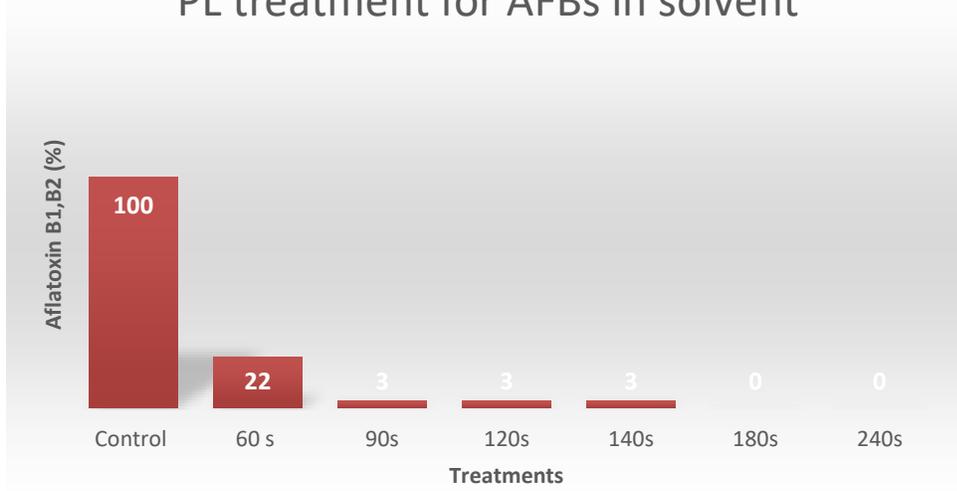


Figure 3-4. The AFB₁, AFB₂ percentages of Pulsed Light treated pure AFB₁, AFB₂ mixture in solvent.

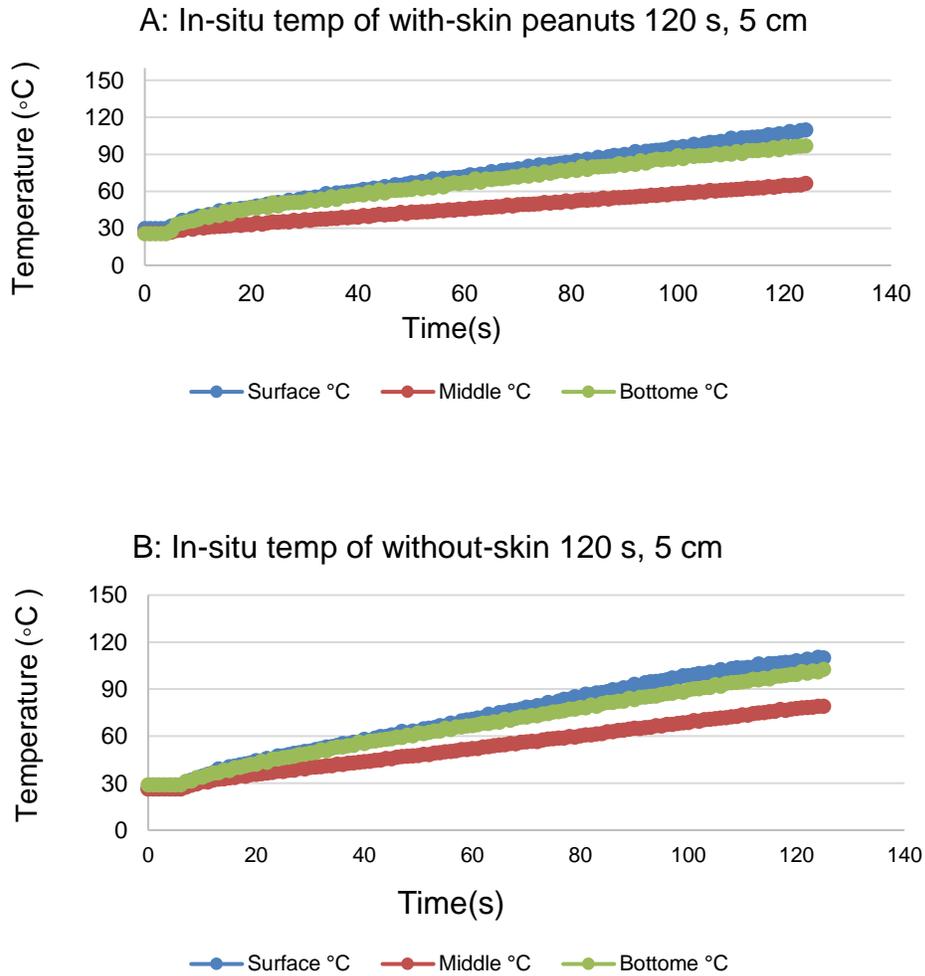
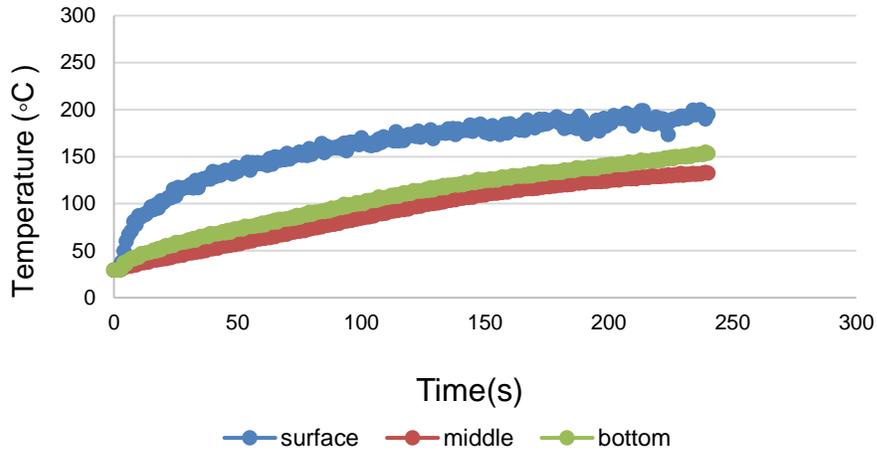


Figure 3-5. The instantaneous temperature measurements. A), B), C), and D) Temperature measurement during PL running: for with-skin and without-skin peanuts each second: A and B for 120 s; C and D for 240 s. Sensors were inserted at the top, in the center, and at the bottom of the grain at 5 cm distance from the strobe.

C: In-situ temp of with-skin peanuts 240s, 5cm



D: In-situ temp of without-skin 240 s, 5 cm

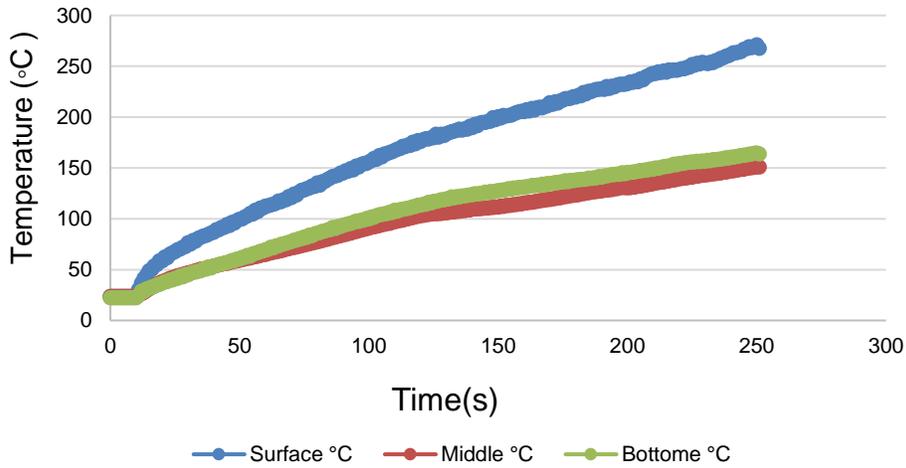
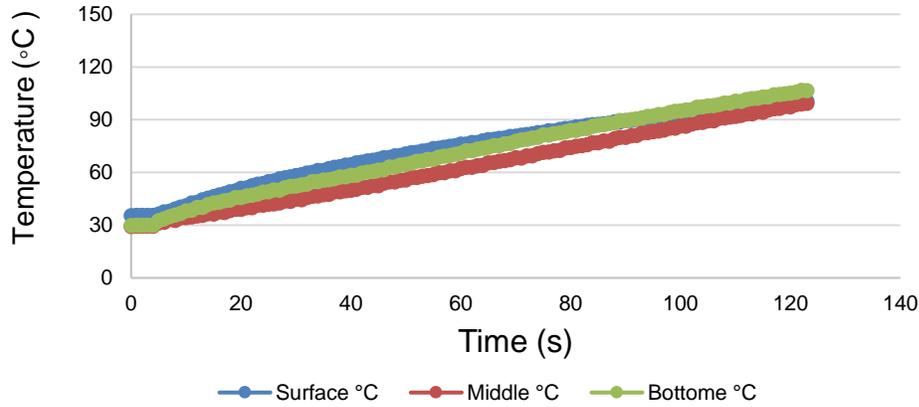


Figure 3-5. Continued.

A: In-situ temp of with-skin peanuts 120 s, 7 cm



B: In-situ temp of without-skin peanuts 120 s, 7 cm

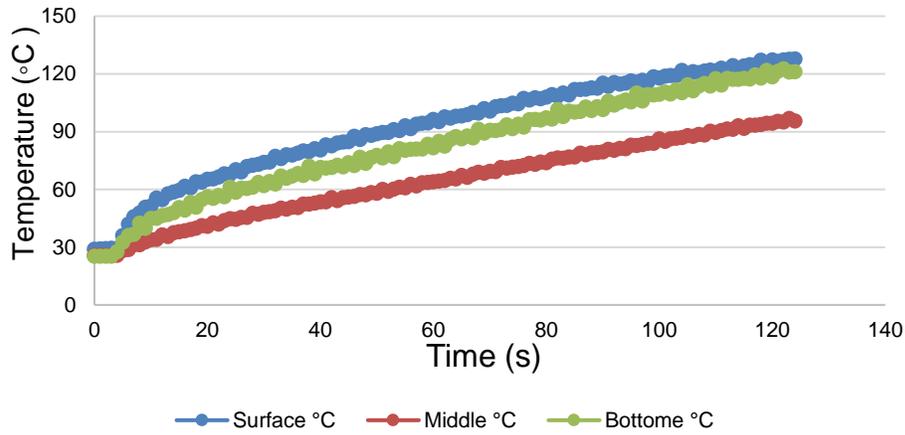
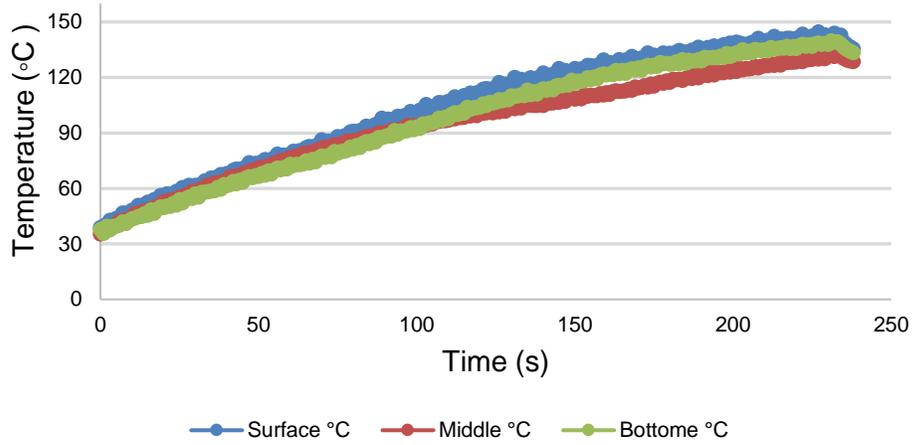


Figure 3-6. The instantaneous temperature measurements. A), B), C), and D) Temperature measurement during PL running: for with-skin and without-skin peanuts each second: A and B for 120 s; C and D for 240 s. Sensors were inserted at the top, in the center, and at the bottom of the grain at 7 cm distance from the strobe.

C: In-situ temp of with-skin peanuts 240 s, 7 cm



D: Without-skin 240 s 7 cm

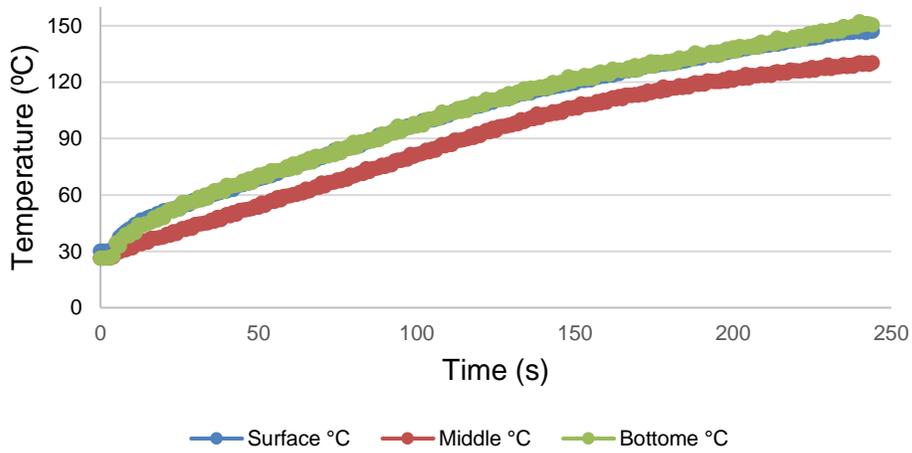


Figure 3-6. Continued.

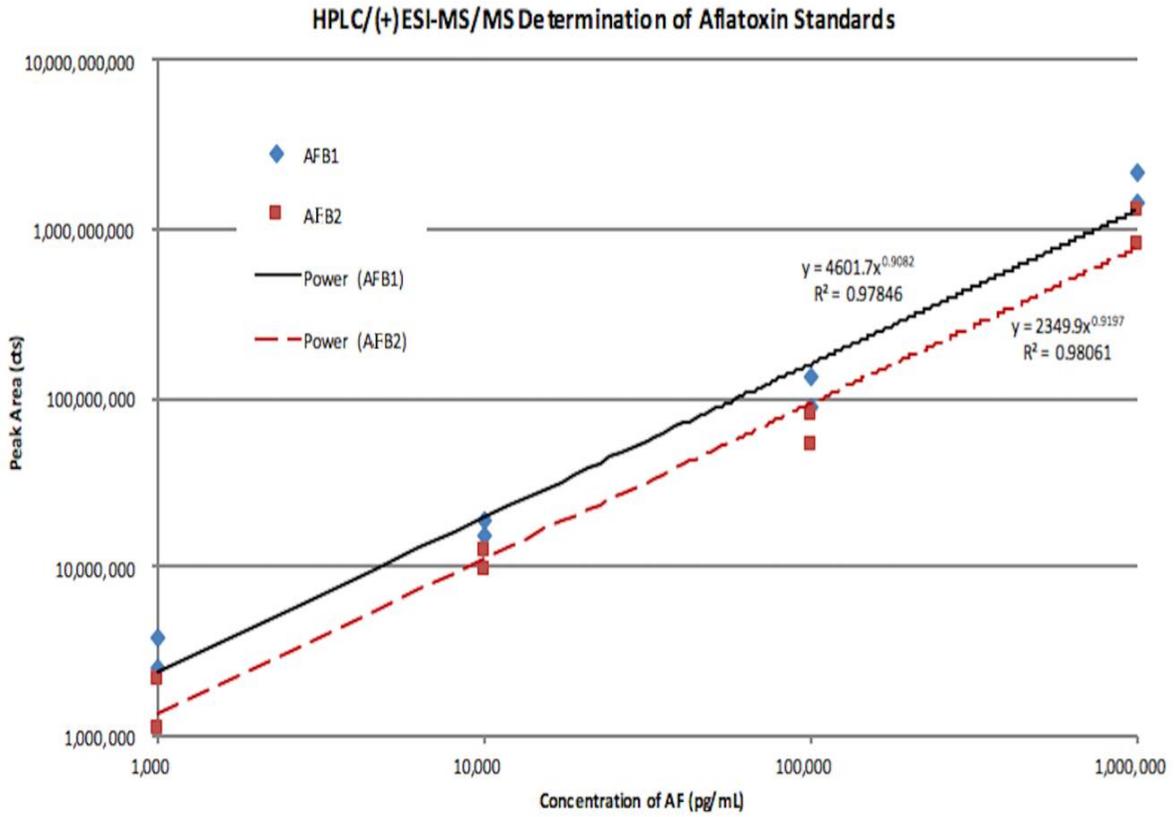
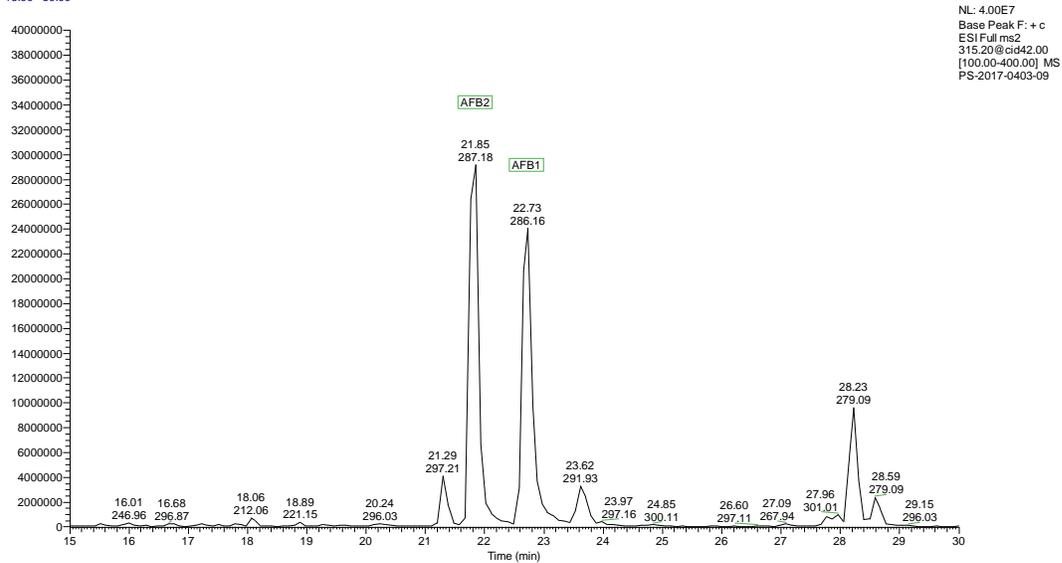


Figure 3-7. LC-MS/MS calibration curve for the AFB₁, AFB₂ standards.

A

RT: 15.00 - 30.00



B

RT: 15.00 - 30.00

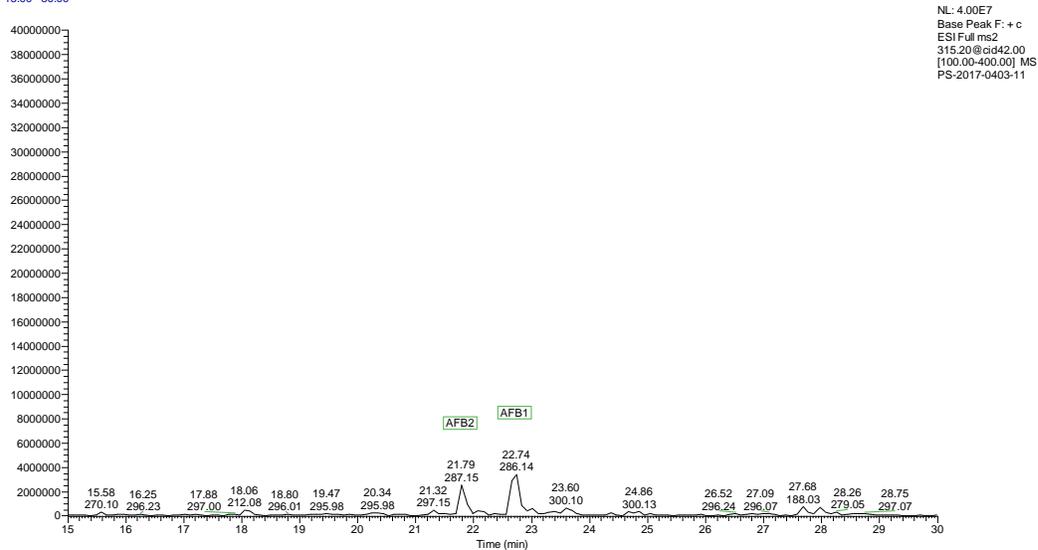


Figure 3-8. LC/MS/MS chromatograms for peanuts. (A) before PL treatment (control), (B) after PL treatment for 240 s at 7 cm.

CHAPTER 4 OPTIMIZATION OF THE PULSED LIGHT (PL) EXPOSURE METHOD FOR AFLATOXIN DETOXIFICATION FOR WITH-SKIN AND WITHOUT-SKIN PEANUTS

Pulsed light is a powerful technique that applies intensive flashes of white light, including broad-spectrum with wavelengths ranged from 200 nm in the ultraviolet region to 1100 nm near-infrared region. The power of each pulse is higher (about 20,000 times) than the power of the sunlight at sea level. The pulse continues for a few hundred millionths of a second and can produce a high-power pulse in this negligible time. Thus, PL has been successfully used to sterilize and eliminate fungi and bacteria in food. The killing ability of PL is four to six times greater than that of the continuous UV light at equal energy levels. The advantages of using PL is that PL light can increase the quality and the shelf life of foods without carrying significant sensory changes (Chung et al., 2008; Dunn et al., 1995; Gómez-López et al., 2007). The sun's photo-catalytic activity is known to be responsible for breaking down many and various pollutants. The specific PL decontamination mechanisms have not been fully elucidated yet, but the most recent hypothesis is that the intensive power of PL is due to the rich broad-spectrum used, and its extreme high power peaks in a very short time, particularly the UV part of the spectrum and its photo-chemical and photo-thermal effects. This photo effect is attributed to the alteration of UV photons into energy, triggering local, sharp, and short temperature elevation at the surface of the treated object. Although the PL has a low penetration level (2 mm from the surface of solid food), this technique effectively changes the structure of the molecules which absorbed the UV light; suggesting that this technique could also be effective in detoxification and

changing the structure of mycotoxins in addition to being active on micro-organisms, especially mycotoxins concentrated at the surface of plants (Moreau et al., 2013).

Aflatoxin(s) (AFTs) are the secondary metabolites of some *Aspergillus* species such as *Aspergillus flavus* and *Aspergillus parasiticus*. Since the fatal effects of AFTs have been discovered, 8,000 published research studies have investigated their exposing acute and chronic toxic, carcinogenic, and mutagenic effects. In addition, at the end of the 1960s, the toxigenic and the carcinogenic potency of AFTs became an emergency matter demanding more research, since the acute hepatotoxic disease (X diseases) struck 100,000 of turkey's lives (Eaton & Groopman, 2013). Several other outbreaks initiated intensive investigations all around the world about AFTs and their potency related to diseases in animals and humans (Eaton & Groopman, 2013). Aflatoxins appeared to have existed for a long time before these outbreaks. However, those dramatic outbreaks grabbed the world's attention to the seriousness of many other diseases that could be attributed to AFT consumption.

Chemically, to understand AFT behavior as a chemical compound, AFT is a lipophilic compound. In the human body, AFT can pass any barrier inside the body and be bioactivated in the organs. Generally, the primary AFT target organ is the liver. This fact has been proven by thousands of studies on AFT toxicity conducted on rodents, poultry, fish, and monkey livers. These animals were fed a diet containing AFB₁. Their livers were analyzed to discover that the damage occurred in the liver after AFT consumption for a specific period. However, not all results were identical since there are fundamental differences in the susceptibility of each species. Furthermore, within the tested species, the level of response was influenced by various other factors such as

their diet, weight, age, sex, exposure to infectious sources, the presence of pharmacological active compounds, and other mycotoxins (Cullen & Newberne, 2013; Zain, 2011).

Pulsed light has been tested on AFB₁ in solution and achieved complete AFT elimination (Moreau et al., 2013). However, PL treatment is known to be affected by the transparency of the treated food. As the transparency increases, the PL penetration increases and the efficiency increases too. As UV absorbance increases, the intensity throughout the product in the reactor decreases and results in a reduction of UV dose delivery (Koutchma, 2009). The mechanisms involved in the decontaminating effects of PL are not clearly known yet, but is probably due to the rich broad-spectrum UV content, short duration, and high peak power delivered to the treated food. PL started to be examined on several types of food after its efficacy was approved in terms of AFT reduction (Méndez-Albores et al., 2005). Therefore, this experiment was conducted to evaluate the efficiency of PL at the range between 100 and 2200 wavelength spectrum on the AFB₁ and AFB₂ detoxification for peanuts which is considered to have big kernels compared with other previously PL treated commodities or foods. The big challenge is treating peanuts practically, given that AFB₁ and AFB₂ are lipophilic compounds. The peanut cell is composed of a 1.2 µm diameter oil-field cavity surrounded by a double membrane. The cell membrane is not permeable for oil but is able to pass water. Thus, the toxins which are inside this cavity will be hard to treat by PL or any other treatment (Figure 4-1).

Materials and Methods

Locally purchased (Publix, Gainesville, FL) with-skin and without-skin runner raw peanuts prepared to be exposed to the PL light. All used tools, materials, and labware

were sanitized by rinsing with 200 µg/mL chlorinated water, immediately followed by rinsing with sterilized water as previously done by Potrebko & Resurreccion (2009). Peanuts were autoclaved and inoculated with a specific strain of *A. flavus* (ATCC® 16875™) as mentioned in Chapter 3. This strain produces AFB₁, AFB₂ in satisfactory levels (Wei & Jong, 1986). Peanuts were then incubated at optimum conditions for fungi growth and AFT production for a sufficient time (21 d), moisture was optimized to 16% and peanuts kept in sealed plastic bags and stored in a -18°C freezer until analysis.

PL Exposure

The PL system consists of three major components: the power supply, the pulse configuration, and the lamps. Energy is stored in a high-power capacitor for a comparatively long time (a fraction of a second), then released to a xenon lamp within a shorter time (nanoseconds to milliseconds). The Xenon lamp was specially designed for this technique. The intensive energy the lamps deliver, produces powerful pulses of light that lasts a few hundred microseconds. These pulses focus on the treatment area.

From the previous work's results (Chapter 3), a major challenge was to achieve a homogenized treatment for peanut samples and increase both the uniformity in treatment and the maximum AFT reduction at the same time. Thus, peanuts need to be rotated or moved during PL treatment, especially at short distances. Therefore, five PL exposing methods (in-plate, sliced peanuts, in-ice tray, rotating in a test tube, and shaking) were examined to achieve best color uniformity, besides greatest AFT reduction in shorter time.

Experimental Design

The inoculated peanuts were prepared as mentioned in Chapter 3. However, this time, five exposing methods were used for without-skin samples and four methods for with-skin samples.

In-plate samples

Samples of 5 g of with-skin and without-skin peanuts were placed in an aluminum plate. To fulfil better results, the appropriate amount of whole peanut kernels was placed in a plate having a relatively small size (7.2 cm radius) given that the PL machine used for this experiment has the highest power intensity directly under the center of the Xenon lamp.

Shaking treatment

A shaker device was built in the lab by connecting an appropriate size iron tray to a lab shaker (MTS 2/4 D S1, IKA, Wilmington, NC) by two long metal rods to provide flexibility and fixing the tray to the shaker body tightly at the same time (Figure 4-14). Peanuts were placed in the middle of this tray and the tray was inserted inside the PL machine to be right under the quartz window. Activating the shaker made the peanuts flip and rotate and shake during exposure to the light (Figure 4-12, 4-13). In this treatment, the only distances used were 7, 10 cm due to the non-suitable height of the machine chamber to house the shaker at 5 cm.

Sliced peanuts

Peanut kernels were cut in slices to improve the UV penetration, given that the penetration of PL is very limited to a few millimeters, and the peanut kernels are relatively thicker than the suggested penetration distance. Slices were then placed in aluminum plates and treated by PL.

In-ice tray treatment

To understand the effective power of PL treatment, and whether the destruction is the action of the photo-chemical of the UV photons or from the heat due to UV photo-thermal and infrared region, peanut plates (6 cm diameter) were placed in larger plates (7.5 cm diameter) that contained ice surrounding all the plate's sides except the upper side. These samples were treated for 30, 60, 90, 120, 180, 120, and 240 s and at distances of 5, 7, and 10 cm (Figure 4-15).

In-tube treatment

Zhao (2013) in her research made a motor, which was built in the Processing Lab of the University of Florida. The motor was fixed to steel plate with a control panel, metal rod, and wires (Figure 4-16). The steel rod rotates carrying a regular glass tube (Fisher Scientific, Rockford, IL) full of peanuts. Once the motor is turned on, the device flips the peanuts inside the test tube while exposing to PL.

The same method was used in this experiment. Five grams of peanuts were placed in a transparent glass test tube. A hole was made at the bottom of the test tube to release the vapor pressure to prevent the tube from exploding. This glass tube was capped by a suitable rubber cap tightened to the edge of the metal rod, which was positioned at the top of the motor. The motor was placed on the PL machine's conveyor belt and adjusted to make sure that the test tube was located directly under the center of the light source (Figure 4-16) (Zhao, 2013). The distances used here were 7, 10 cm, since the distance of 5 cm wasn't suitable for the device's height.

Treatments used a 7x3 factorial design; the treatment factors were PL exposure times of 30, 60, 90, 120, 180, 120, and 240 min and distances of 5, 7, and 10 cm.

Samples were treated in triplicate. Each treatment used three 5-g samples treated separately.

After PL treatment, samples were ground in a coffee grinder (Model LXC-150, 50/60Hz, 180W, Keunex, Korea), then extracted by mixing and blending in a lab blender (31BL91, Waring, Dynamics Corporation of America, Hartford, CT) for 2 min with methanol 70% and filtered using Whatman number 1 filter paper as in Chapter 3.

The extracted samples were analyzed using ELISA (Agra Quant® ELISA Aflatoxin Kit, Romer Labs, Getzersdorf, Austria). The kit included five different AFT concentration standards 0, 4, 10, 20, and 40 ppb to create a standard calibration curve to calculate AFB₁ and AFB₂ concentrations for all PL treated samples and compare the treated samples AFT concentration with the positive control (fungi inoculated peanuts without PL treatment).

Chromatographic analyses were performed on samples that had the highest reduction percentage to confirm the ELISA results by using HPLC/MS/MS instrument (the same as Chapter 3).

The highest treatment of all method were statistically compared using 2016 Microsoft Excel software; factorial analysis one-way ANOVA. Means and standard divisions were calculated. The mean significant differences were obtained using the least significant differences (LSD) procedure.

Results and Discussion

Pulsed light treatment is strongly affected by the transparency of the treated food. The colored or the opaque foods have UV light penetration capacity less than transparent food such as water and some juices (Guerrero-Beltran & Barbosa-Canovas, 2004). When the transparency increases, the UV penetrates deeply inside the food

matrix permitting a complete decontamination for the contaminant (Feuilloley et al., 2006). Wallen & Fraenkel (2001) implied that the effect of PL is limited to the first few millimeters from the surface of the solid food. Thus, it is necessary to control the thickness of irradiated foods due to lower UV light penetration (to several millimeters depending upon their optical properties). Pulsed light easily penetrates water but difficultly passes through milk and other turbid foods. Granular or opaque foods such as peanut need to be presented as a thin layer or constant shaking or moving during the UV light detoxification (Diao et al., 2015). In addition, expanding the penetration capacity of UV light is an essential step to improve its detoxification efficiency. Therefore, many PL exposing methods were investigated in this chapter to fulfil the best AFT reduction with respect to peanut nutrient and sensory properties.

ELISA and HPLC-MS/MS Results

With-Skin Samples

In-plate treatment

After PL treatment, AFT concentrations in peanut kernels showed reduction depending on the treatment method, exposure time, and the distance between samples to the Xenon lamp. The presented study results revealed that PL treatment can reduce AFTs. This reduction had a strong positive correlation coefficient to the temperature elevation and treatment time (Table 3-5, 3-8). However, it had a negative correlation coefficient to the distance for both with-skin and without-skin peanuts in all treatment methods (Figure 4-2). The highest reduction (82%) was achieved after treatment for 300 s at 5 cm distance from the PL's strobe and with increasing the treatment time the reduction would increase. However, increasing the treatment time above 300 s accompanied with decreasing the distance led to some burn to the upper surface of the

peanut kernels during in-plate treatment causing a degradation in peanut quality. LC-MS/MS results agreed with the ELISA results. The reduction reported for AFB₁, AFB₂ for treatment duration 240 s at 7 cm were 87.2% and 84.7%, respectively.

Shaking treatment

Shaking treatment was the best in terms of achieved a high reduction percentage reach to 86% after 240 s treatment (Figure 4-3) of PL with maintaining good peanut quality. There is a possibility of having better reduction with keeping the peanut sensory quality by increasing treatment time with respect to the amount of light absorbed by the food. LC-MS/MS results agreed with the ELISA results. The reduction reported for AFB₁, AFB₂ for treatment duration 240 s at 7 cm were 91.3% and 88.5%, respectively.

In-ice treatment

This treatment conducted to avoid the temperature elevation which could affect peanut quality. At the same time this treatment examined the photo effect solo, without the temperature effects. The highest temperature recorded in this treatment was 107°C in the 300 s treatment (Figure 4-4). Results showed that reduction achieved in this treatment was much lower than all other treatments. This demonstrates that the PL treatment led to mostly photo-thermal destruction. Strictly photo reduction (temperature controlled treatment) caused a low level of AFTs (AFB₁, AFB₂) reduction which at maximum was 50% reduction. The LC-MS/MS results showed lower reduction than the ELISA results. The reduction reported for AFB₁, AFB₂ for treatment duration 300 s at 7 cm were 28% and 65%, respectively.

In-tube treatment

Peanuts were treated by placing in a glass test tube and fixed to a rotating motor to provide treatment from all sides and the provide uniformity in the treatment and avoid

the high increase in temperature during treatment. Results weren't as good as the former treatments (using the same distance and time) in terms of AFTs (AFB₁, AFB₂) reduction, which reached to 52.4% when treated for 300 s (Figure 4-5). LC-MS/MS results were higher from ELISA results. The reduction reported for AFB₁, AFB₂ for treatment duration 400 s at 7 cm were 86.5% and 93.2%, respectively.

Factorial statistical analysis one-way ANOVA was conducted to determine the significant effects of the differences in the four exposing methods during PL treatment on the AFT reduction percentages, and on temperature elevation (Figure 4-2, 4-3, 4-4, 4-5). The highest treatment of all the four exposing method of the with-skin samples were statistically analyzed. Results were significantly different at $\alpha = 0.05$ confidence interval between the in-plate treatment, in-ice treatment, and in-tube treatment. However, the in-plate treatment wasn't significantly different with the shaking treatment.

Without-Skin Samples

In-plate treatment

Peanuts without-skin were more sensitive to PL treatment. All methods recorded best reduction percentage for without skin peanuts due to the skin which acted as a barrier. The skin likely shaded the kernels and decreased the effect of PL. In addition, the dark color of the skin will increase the light absorbance on the surface not allowing it to penetrate sufficiently. Therefore, the highest reduction achieved (90.9%) was in without-skin peanuts treated by PL treatment without reducing the quality of the kernels for 240 s at 5 cm. Increasing the treatment time above 300 s (Figure 4-6), accompanied with decreasing the distance, led to some burning and uniform dark spots at the top of the peanut surface. LC-MS/MS results agreed with the ELISA results. The reduction

reported for AFB₁, AFB₂ for treatment duration 240 s at 7 cm were 90.3% and 95.2%, respectively.

Shaking treatment

Shaking treatment was the best in terms of achieving a high reduction percentage reaching to 91.7% after 240 s of PL treatment at 7 cm to the quartz window, accompanied with saving the quality of peanuts and resulted in semi-roasted peanuts. There is a possibility of having better reduction with keeping the peanut sensory quality by increasing treatment time with respect to the irradiation absorbed by food. LC-MS/MS results agreed with the ELISA results. The reduction reported for AFB₁, AFB₂ for treatment duration 240 s at 7 cm (Figure 4-7) were 94.1% and 93.9%, respectively.

Slices treatment

Peanuts were sliced to get better UV penetration treatment. Results were good and the reduction reached to 82% in 210 s, and the temperature reached 170°C (Figure 4-8). However, the quality of the treatment wasn't sufficient to be presented to consumers. This treatment ended with fast burning of the edges of the peanut slices.

In-ice treatment

This treatment was conducted to avoid the temperature elevation, which could affect the peanut's quality, and at the same time examined the photo effect solo without the temperature effects. The highest temperature reached was 126°C (Figure 4-9). Results showed that reduction achieved in this treatment was much lower than all other treatments by means that the PL treatment has photo-thermal destruction; thus, the photon, without increasing in temperature, caused a low level of AFTs (AFB₁, AFB₂) reduction which was 49.1%. LC-MS/MS results were lower than ELISA results. The

reduction reported for AFB₁, AFB₂ for treatment duration of 300 s at 7 cm was 28% and 65%, respectively.

In-tube treatment

Peanuts were treated by placing in a glass test tube and fixed to a rotating motor to treat all the peanuts' sides and provide uniformity in the treatment and color, in addition to avoiding a high increase in temperature during treatment. Results were not as good as the former treatments without rotation (using the same distance and time) in terms of AFTs (AFB₁, AFB₂) reduction, which reached to 63.16% when treated for 300 s (Figure 4-10). LC-MS/MS results were higher than ELISA results. The reduction reported by LC-MS/MS for AFB₁, AFB₂ for treatment duration 400 s at 7 cm were 75.2% and 78.6%, respectively for the without skin treatments.

Factorial statistical analysis one-way ANOVA was conducted to determine the significant effects of the differences in the exposing methods during PL treatment on the AFT reduction percentages, and on temperature elevation (Figure 4-6, 4-7, 4-8, 4-9, 4-10). The highest treatment of all the five exposing methods for the without-skin samples were statistically analyzed. Results were significantly different at $\alpha = 0.05$ confidence interval between the in-plate treatment, in-ice treatment, and in-tube treatment. However, the in-plate treatment wasn't significantly different with the shaking treatment and the slice treatment.

PL

Pulsed light originates from the same principle as UV decontamination. PL is quite close to the UV treatment, but PL has higher intensity and greater efficiency than UV decontamination techniques because PL amplifies the photo-catalytic activity of light, which is naturally responsible for extirpating a variety of pollutants. Various

mechanisms have been proposed to explain the lethal effects of PL, all of them related to the UV part of the spectrum and its photo-chemical and photo-thermal effects (Elmnasser et al., 2007; Gómez-López et al., 2007). The photo-chemical mechanism involves the UV part. However, this study's results indicated that the AFT degradation was the result of two accompanied PL effects: the photo-thermal and the photo-chemical effect. Infrared heat can also contribute to the AFT destruction effect (Yang et al., 2012). Therefore, PL is considered a fast method to transfer a large amount of photo-thermal energy to the product's surface.

An infrared thermometer was used to measure the increasing of temperature after all treatments and compared with the initial temperature (room temperature 23–25°C). The highest temperature reached among all treatments was 180°C for the in-plate treatment. However, the AFTs have high decomposition temperatures ranging from 237°C to 306°C, and solid AFB₁ is quite stable to dry heating at temperatures below its thermal decomposition temperature of 267°C (Rustom, 1997). Thus, in the present study, the heat elevation should not have been high enough to destroy AFTs thermally. Generally, PL treatment, accompanied with manipulating time and distance and shaking the samples, fulfilled a high reduction at a relatively low temperature.

Surrounding the samples with ice was conducted to examine the effect of the photo-chemical effect in solo on the AFT degradation. However, all the results showed lower reduction percentages compared with other treatments. On the other hand, treating the peanuts (the whole kernels) inside rotating test tube (Figure 4-18) showed a low reduction percentage because of the layer of glass which worked as a barrier to prevent part of the light from reaching the samples. However, the quality of the

produced samples was perfect in terms of the distribution of the roasting color. Thus, this treatment could be used for a longer time with respect to the increasing of the power expenses and time, which will be the main disadvantages of this method.

Zhao (2013) used the rotating tube method to provide uniform PL treatment to inhibit the allergenic effects of peanuts by destroying proteins responsible for these allergic symptoms in human. Zhao's results were promising since using this device increases the PL treatment time, allowing the peanuts to absorb as much as available of the effective spectrum, avoiding the quality deterioration of peanuts, and allowing longer treatment than in-plate treatment (Zhao, 2013).

Table 4-1. LC-MS/MS analysis for 5g without-skin peanuts with different PL treatments.

Treatments	R% AFB ₂	R% AFB ₁
In-Plate	95.2a	90.3a
Shaking	93.9a	94.1a
Tube	78.6b	75.2b
Control	0	0

R%: reduction percentage, PL treatment: 240 s at 7 cm distance from the strobe. Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis

Table 4-2. LC-MS/MS analysis for 5 g with-skin peanuts treated with PL.

Treatments	R% AFB ₂	R% AFB ₁
In-Plate	84.7a	87.2a
Shaking	88.5a	91.3a
Tube	93.2a	86.5a
In-ice	65.0b	28.0b
Control	0	0

R%: reduction percentage, PL treatment: 240 s and 7 cm distance from the strobe.

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis

Table 4-3. Peroxide value (meq of peroxide/L) of peanuts oil after PL treatments of peanuts.

Treatment	Control	240 s-Shaking	240 s-Plate	300 s-Shaking	300 s-Plate
240 s/ 5 cm	1.30 ± 0.14a	0.43 ± 0.05c	0.96 ± 0.05c	0.67 ± 0.06bc	0.83 ± 0.03c
240 s/ 7 cm	1.10 ± 0.14a	0.50 ± 0.04c	0.89 ± 0.04c	0.40 ± 0.07b	1.20 ± 0.26c

* Mean ± standard deviation (n = 3). Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD for mean differences analysis.

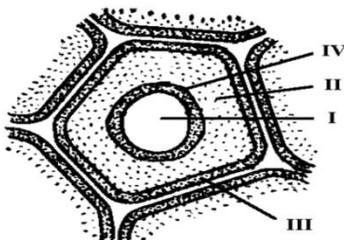


Figure 4-1. The model structure of peanut cells:

I = cell cavity; II = amorphous matrix;

III = external membrane film; IV = internal membrane film (Zakhartchenko et al., 1998)

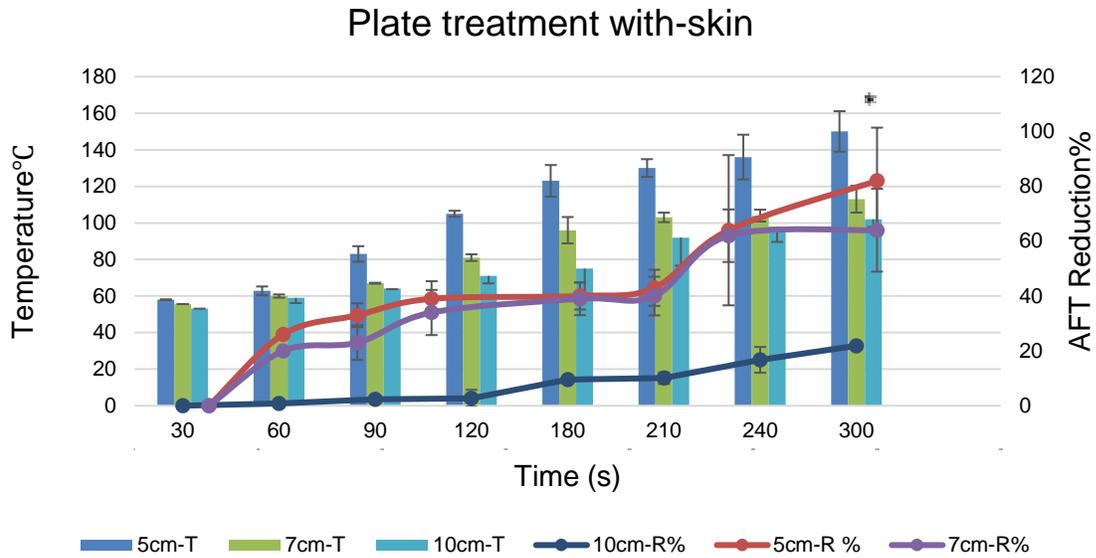


Figure 4-2. Reduction percentages and temperature measurements of in-plate PL treated peanuts. Three different distances (5, 7, and 10 cm) were used for with-skin peanuts.

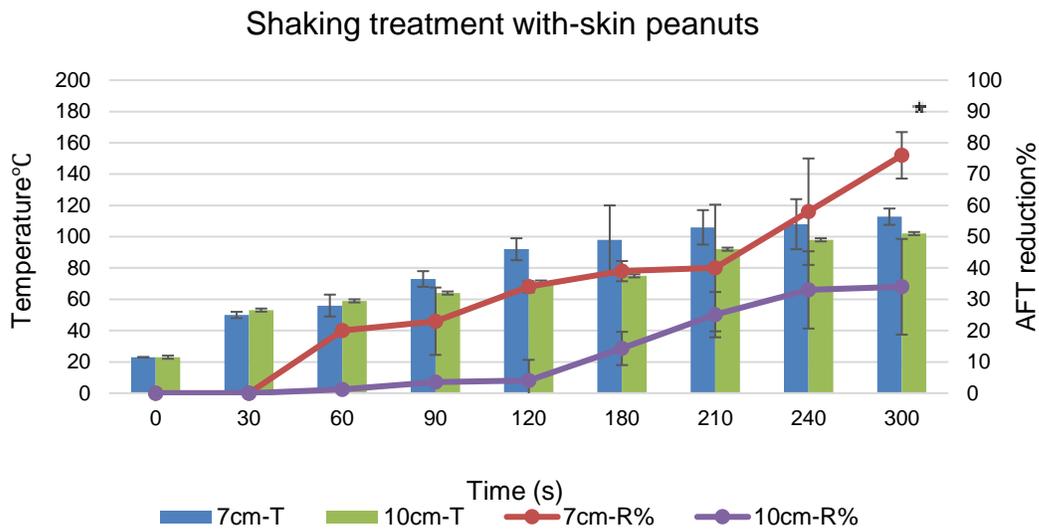


Figure 4-3. Reduction percentages and temperature measurements of shaking PL treated peanuts. Two different distances (7, 10 cm) were used for with-skin peanuts.

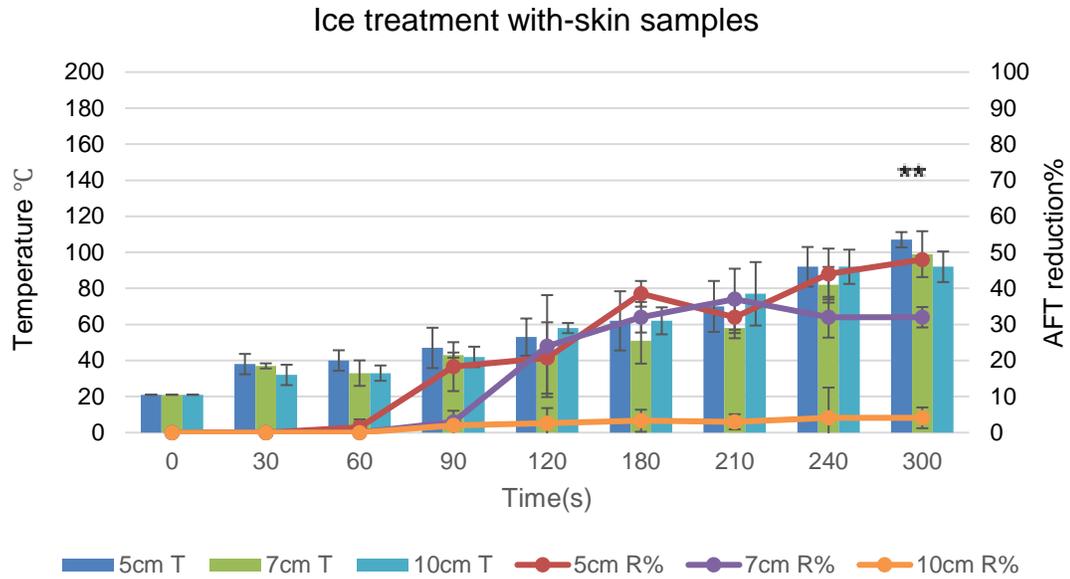


Figure 4-4. Reduction percentages and temperature measurements of in-ice PL treated peanuts. Three different distances (5, 7, and 10 cm) were used for with-skin peanuts.

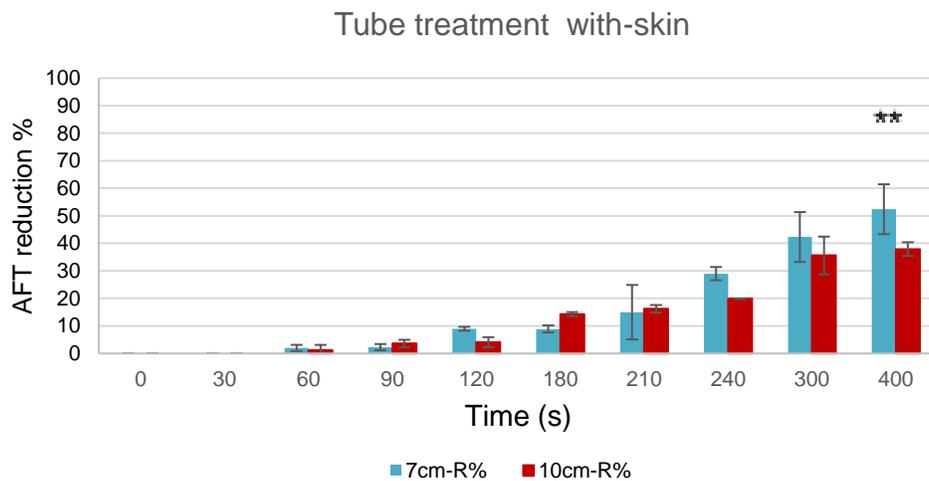


Figure 4-5. Reduction percentages and temperature measurements of in-tube PL treated peanuts. Two different distances (7, 10 cm) were used for with-skin peanuts.

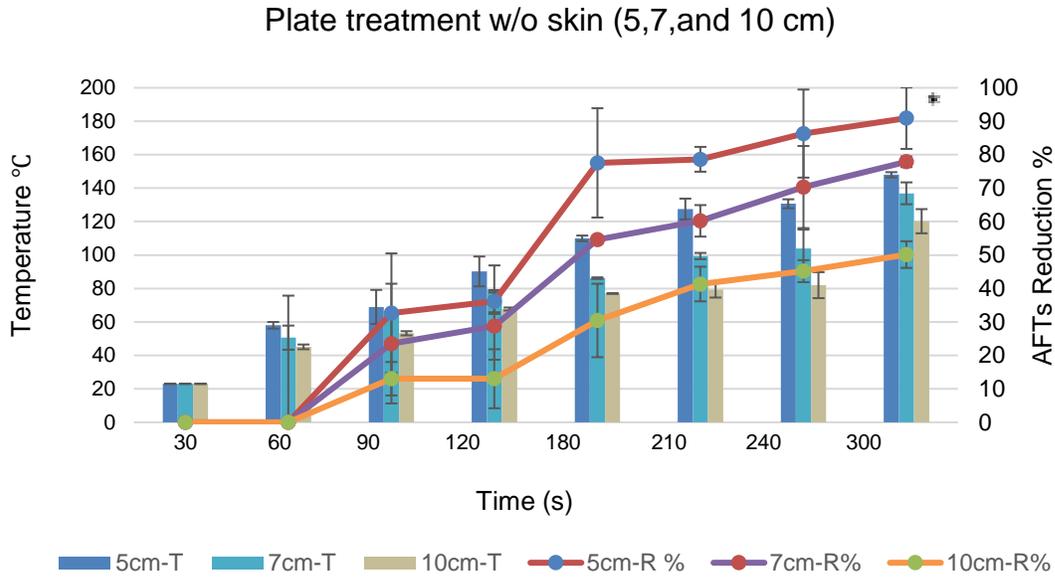


Figure 4-6. Reduction percentages and temperature measurements of in-plate PL treated peanuts. Three different distances (5, 7, and 10 cm) were used for w/o peanuts.

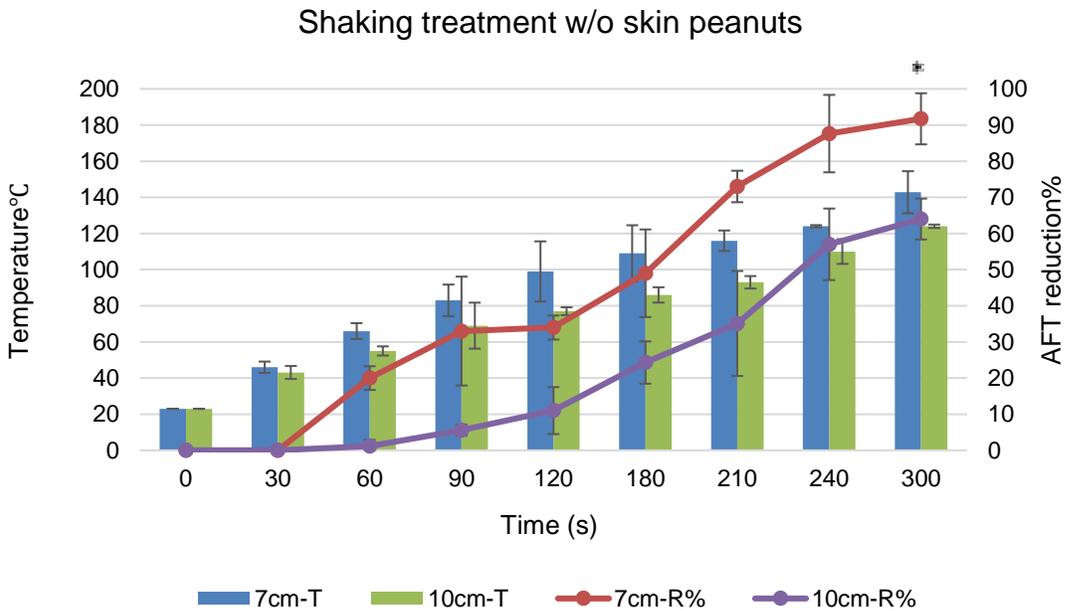


Figure 4-7. Reduction percentages and temperature measurements of shaking PL treated peanuts. Two different (7, 10 cm) were used for w/o skin peanuts.

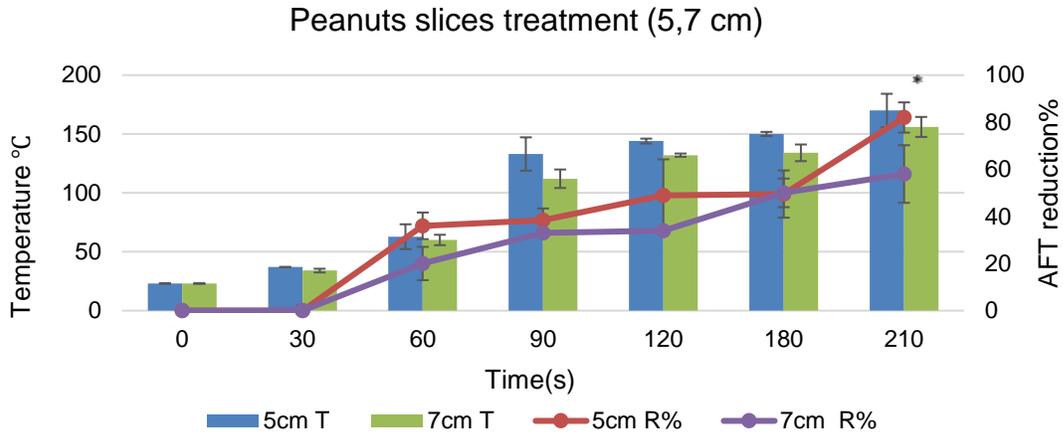


Figure 4-8. Reduction percentages and temperature measurements of slices PL treated peanuts. Two different (7, 10 cm) were used for w/o skin peanuts.

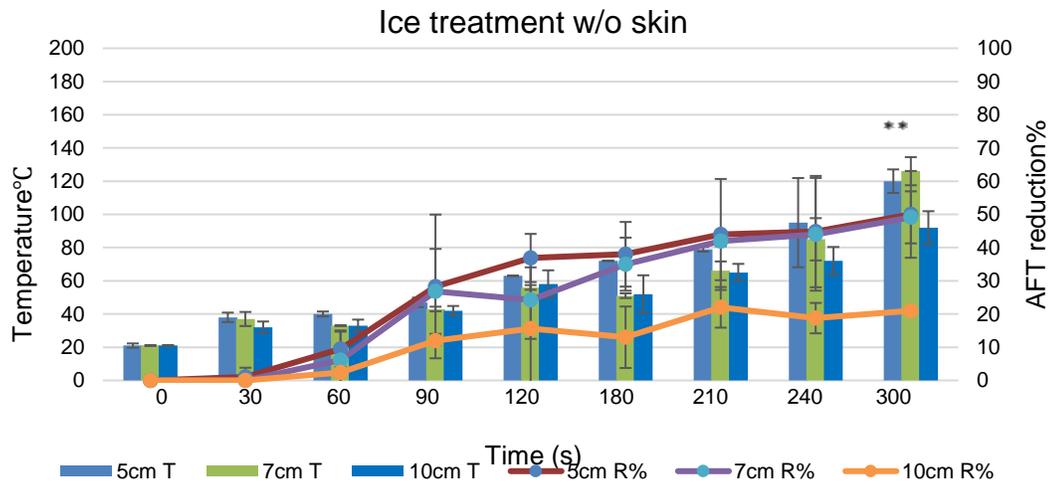


Figure 4-9. Reduction percentages and temperature measurements of in-ice PL treated peanuts. Three different distances (5, 7, and 10 cm) were used for w/o skin peanuts.

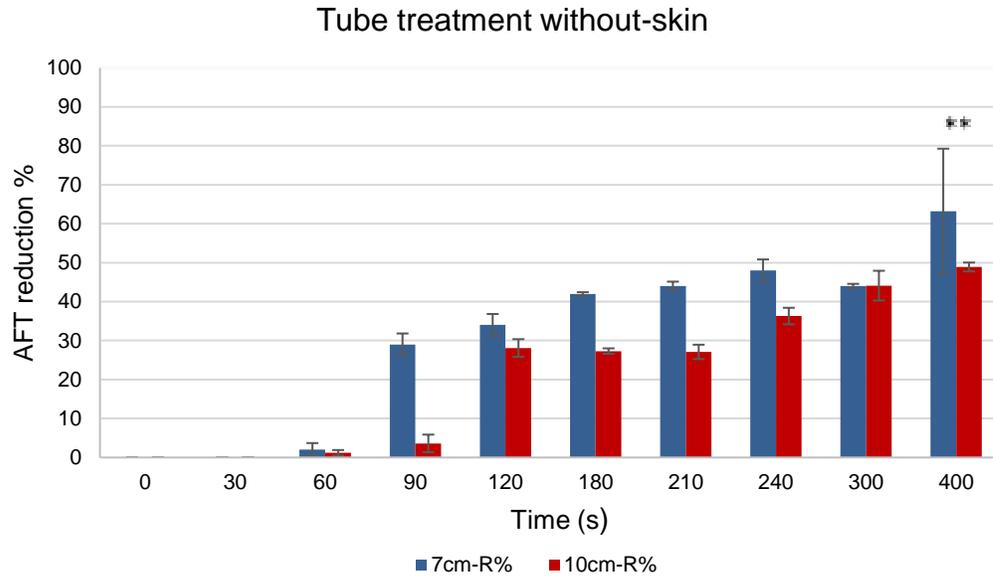


Figure 4-10. Reduction percentages and temperature measurements of in-tube PL treated peanuts. Two different distances (7, 10 cm) PL treatment for w/o skin peanuts.



Figure 4-11. In-plate PL treatment for peanuts. (Photo courtesy of author).

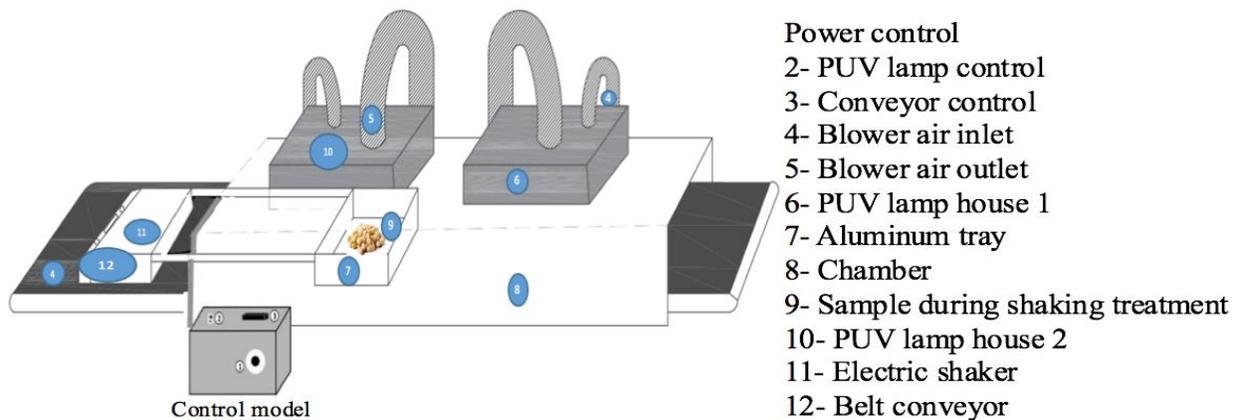


Figure 4-12. Schematic representation of Shaking PL treatment. (Photo courtesy of author).



Figure 4-13. Shaking device inserted inside the PL machine. (Photo courtesy of author).

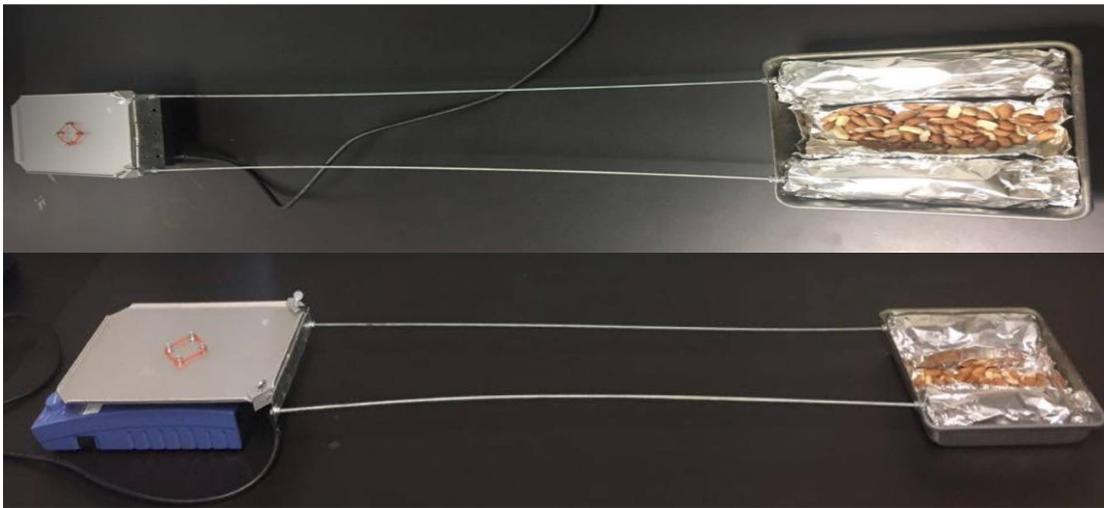


Figure 4-14. Shaking device for shaking treatment for PL treatment of peanuts. (Photo courtesy of author).



Figure 4-15. Double dishes for In-ice PL treatment of peanuts. (Photo courtesy of author).

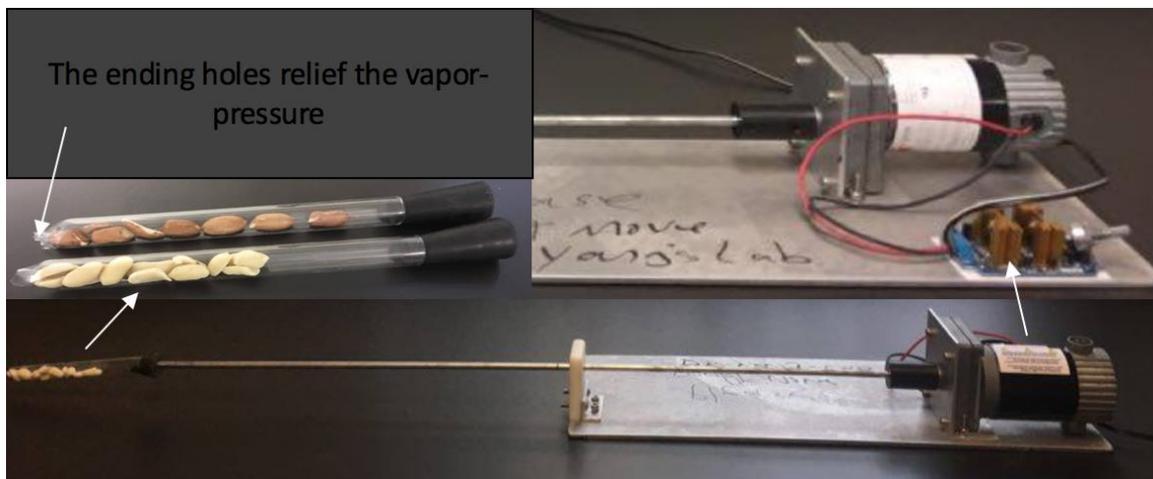


Figure 4-16. Rotating motor for In-tube rotating PL treatment of peanuts. (Photo courtesy of author).

CHAPTER 5 INACTIVATION OF AFLATOXINS B₁ AND B₂ IN PEANUTS BY COMBINING TWO TREATMENTS: PULSED LIGHT (PL) AND CITRIC ACID

The AFTs are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*. There are 18 different identified types of AFTs. Nevertheless, the major members are AFT B₁, B₂, G₁, and G₂. AFTs are coumarin derivatives with a fused dihydrofurofuran moiety (Gupta, 2012; Iram et al., 2016). They are potent toxigenic, carcinogenic, and immunosuppressive compounds commonly found in peanut and associated products. Chronic exposure to low levels of AFB₁ with the presence of hepatitis B virus could cause a serious development of hepatocellular carcinoma in the human body. In addition, the International Agency for Research on Cancer (IARC) announced aflatoxin B₁ as a group 1 carcinogen to humans in the Sixth Annual Report on Carcinogens in 1991 (National Toxicology Program, 2011). Since then, AFTs have been confirmed as dangerous contaminants causing acute and chronic effects. The health and science society has intensified their effort to find a way to get rid of this risk which has threatened people's lives through their food.

Numerous methods for reducing AFTs have been investigated since the 1960s: chemical, physical, and biological approaches. Most of these methods have some disadvantages over the favorable attributes in human edible substances. Some points should be taken into consideration as a nominal requirement: the safety of the food after treatment, the used method should eliminate AFTs to at least the FDA requirement (≤ 20 ng/g) (FDA, 1994; Mobeen, 2011), treatment shouldn't leave any unfavorable or toxic residual, and finally it should maintain food quality. The chemical approach for AFT detoxification in food in mild conditions was one of the scientist-favored methods, since the treatment will not lead to nutrient value degradation because of temperature

elevation. Therefore, several chemicals have been tested through the last four decades for AFT destruction in several types of commodities. Most of them achieved a remarkable reduction. However, most of them carried out the concerns of the possible toxic residual production and sensory damaging attributes. Recently, ozone and ammonium treatments have been used widely to destroy AFTs in water, peanut meal, cottonseed, and maize, especially for animal feed (Rustom, 1997).

Pulsed ultra-violet Light (PL) is one of the proposed new technologies which is presented as non-thermal food treatment regarding microbial inactivation by using broad spectral wavelengths (200 to 1100 nm), including UV spectrum. Pulsed light has proven its capability of destroying and reducing several types of harmful compounds, including bacteria, viruses, enzymes, allergens, and toxins.

In high intensity continuous UV treatment, the photo-degradation rate of AFB₁ is strongly affected by UV intensity. Only few literatures showed the same UV intensity (Liu et al., 2011). AFB₁ (2 mg/kg) in peanut oil can be destroyed completely when exposed to UV irradiation for 30 min at an intensity of 8000 J/cm², while it was degraded by about 85% and 79% at the lower intensity of 4000 and 2000 J/cm², respectively (Diao et al., 2015). However, great irradiation intensity may cause critical degradation in peanut quality. Therefore, the intensity and exposure time of the UV irradiation must be controlled to keep the safety and quality of irradiated foods (Diao et al., 2015). The pulsed intensive light is 20,000 times more than the continuous light. In the presented experiment, PL was used to degrade AFTs in peanuts. The exposure times and distances were investigated from all aspects to provide an optimum treatment for the best AFB₁, AFB₂ reduction and keeping good quality to the product. The used pulsed

light delivered 1.27 J/cm² to the peanuts at 1.9 from the strobe. Results were promising as shown in Chapter 3 and 4. However, complete reduction hasn't been achieved. Therefore, soaking the peanuts in citric acid before PL treatment was investigated in this chapter, as earlier literature declared that low pH enhances the UV efficiency (Diao et al., 2015). Citric acid has been tested before alone for AFT detoxification in duckling feed and rice (Méndez-Albores et al., 2007). Many physical, chemical, biological, and irradiation methods have been explored to decrease or remove AFTs in foods. Pulsed light (PL) and citric acid treatments are two of these techniques. Individually, each treatment showed good results in the degradation of AFTs for some commodities. However, a complete elimination of AFTs was not achieved by either method.

Many current studies concluded that UV wavelength, types of AFTs, irradiation intensity, moisture contents of foods, exposure time, thickness, and pH of irradiated foods drastically affect UV elimination efficacy. AFB₁ is reported to be highly sensitive to UV irradiation at a pH of less than 3 or greater than 10 (Diao et al., 2015). Thus, adding citric acid to PL treatment was tested in this study.

Material and Methods

Sample preparation was the same as Chapter 3. With-skin and without-skin peanuts were inoculated with *Aspergillus flavus* (dominant AFB₁, AFB₂ producer). After a proper incubation period, 5 g of peanuts were soaked in 0.1 N citric acid at a concentration of 2 mL/g for 1 h, as is similar to the method used by Méndez-Albrose (2004). Samples were either washed with distilled water or citric acid solution. Moisture was optimized to 16% as in Chapter 3. Then, PL treatment was conducted (Model# LHS40LMP HSG from Xenon Corp, Wilmington, MA) in a shaking tray for 210 s or 240 s

at 7 cm. AFTs were extracted using methanol/water (70/30). Samples were analyzed using ELISA and HPLC-MS/MS to determine AFB₁, AFB₂ reduction.

Experimental Design and Statistical Analysis

This experiment was conducted in triplicate. After the preparation step as in Chapter 3 and the citric acid treatment for specific samples, each sample was placed in the tray which was fixed to a shaker (MTS 2/4 D S1, IKA, Wilmington, NC) to be exposed to the light from all sides at the same time. Samples were extracted and AFTs were measured by using ELISA and HPLC-MS/MS. Data were analyzed statistically using two-way ANOVA test at 0.05 level of confidence (Microsoft Excel 2016) followed by Fisher Least Significance Differences (LSD) method for means differences to evaluate the significance in differences among different treatments and the control (untreated peanuts) at this confidence interval.

Results and Discussion

ELISA Results

ELISA results showed a reduction percentage of AFB₁, AFB₂ in with-skin and without-skin peanuts with citric acid of about 88.29% and 98.12%, respectively (Table 5-1, 5-2). When citric acid treatment was applied individually, the reduction percentages were 20.6 and 29.3, respectively, for with-skin and without-skin peanuts, and the PL treatment individually resulted in 78.9 and 87.1 for with-skin and without-skin, respectively. It was obvious that the hurdle technique and soaking the peanuts in 0.1N citric acid preceding the PL treatments achieved the best results for AFB₁, B₂ reduction (Table 5-1).

HPLC-MS/MS Results

The LC-MS results revealed that treatment with citric acid individually caused an AFB₁, AFB₂ reduction with washing reached to 12.6% and 51.1%, respectively. However, treating the peanuts with the PL alone led to AFB₁, AFB₂ reduction reaching to 90.3% and 95.2%, respectively. However, the hurdle technique (citric acid) and PL led to AFB₁, AFB₂ reduction reaching to 98.9% and 98.1%, respectively, when treatment was applied without washing the citric acid from the surface of the peanuts (Table 5-2, 5-3). Acid treatments have the ability to alter the chemical structure of AFB, specifically alteration of AFB₁ to AFB₂. The converted substance has mutagenicity 1000 times lower than the original toxin (Rustom, 1997).

Discussion

Aflatoxin B₁ is an invisible food threat. AFT's residue contamination has been reported in peanuts and peanut products. Therefore, a practice to obtain peanuts free of AFTs is important, since peanut consumption has increased in the last century due to its distribution all around the world.

However, any detoxification method intended to be used should not cause any undesirable alterations to the nutritional and organoleptic qualities of the peanuts (Samarajeewa et al., 1990). Previous studies revealed that there was an obvious change in the chemical composition of food components, and some product quality degradation occurred when the UV light treatment is applied at a high dose on some foods (Kolakowska, 2002). However, as shown from other study results, UV irradiation may not cause any alteration effects if UV irradiation is applied in reasonable amounts (Caminiti et al., 2012; Taze et al., 2015; Tripathi & Mishra, 2010).

Therefore, a hurdle technique or combining two methods is essential to decrease the UV exposure time and enhance the output of both treatments. The two techniques we used in this experiment, individually each one of them achieved good but not sufficient results in terms of AFT degradation, and quality retention of the peanuts. Soaking the peanuts in 0.1 N citric acid for 30 min at a lower concentration (2 mL/g) from what Méndez-Albores et al. (2005) used (15 min in 3 mL/g, 1 N citric acid) was more effective than the shaking treatment at 7 cm for 240 s (Chapter 4). Thus, these parameters efficiency were enhanced by the soaking step in 0.1 N citric acid for 1 h.

Comparably, in terms of using chemicals to enhance irradiation treatment, Farah et al. (1983) used an autoclave at 116°C, 0.7 bar to cook raw with-skin peanuts after soaked it in a 5% NaCl solution for 30 min. This treatment showed a good reduction for total content of AFB₁, AFG₁, AFB₂, and AFG₂ by 80-100%. The removal of the toxins was attributed to the addition of NaCl, as compared to unsalted controls (Masimango et al., 1978; Rustom, 1997). In addition, Rastegar (2017) showed that roasting AFB₁ contaminated pistachios with 30 ml water, 15 ml lemon juice and 2.25 g of citric acid at 120 C for 1 h reduced the level of AFB₁ in 49.2 ± 3.5% from the initial level (268 and 383 ng/g) without a noticeable change in desired attributes of pistachios (Rastegar et al., 2017).

Possible Mechanisms of AFT Degradation by PL and Citric Acid Treatments

During peanut PL exposure, three possible degradation mechanisms could have happened to the AFTs: one is the effect of light, since the range of spectrum used in this experiment is included UVA and UVC, which have been proven their ability for the destruction of AFTs due to their photosensitivity. Diao et al. (2015) in his review showed

that exposing to UV at wavelengths 220–400 to several types of food (peanut, peanut oil, coconut oil, red chili powder, walnut, almond, pistachio, and dried figs) resulted in AFT reduction from 45.7% to 100% during different exposure time from 10 to 60 min and light intensity ranged from 64 J/cm² to 8000 J/cm² (Diao et al., 2015). Lillard & Lantin (1970) indicated that the best degradation of AFB₁ by irradiation is at 362 nm. Atalla et al. (2004) and Jubeen et al. (2012) reported that the C8-9 double bond in the terminal furan ring of AFB₁ can be degraded easily by UV light at 362 nm. However, AFB₂ and AFG₂ don't have the C8-9 double bond in their terminal furan ring. Thus, they were easily degraded by UVC (especially 254 nm). However, Basaran (2009) reported that the UV light of 254 nm did not affect AFG₂ and AFB₂ but significantly reduced AFG₁ and AFB₁.

The second possible degradation mechanism is that the effect of citric acids on AFTs. AFB₁ is reported to be highly sensitive to UV irradiation at a pH of less than 3 or greater than 10 (Lillard & Lantin, 1970; Rustom, 1997). Thus, to decrease the pH, 0.1 N citric acid (pH 2.9) for 1 h (3 mL/g contaminated peanuts) was added to peanuts before PL treatment to enhance the UV efficiency. Méndez-Albores et al. (2007) study of treating duckling feed with 1 N (pH 2.4) aqueous citric acid for 15 min (3 mL/g of contaminated feed) showed that feed with initial concentration 110 ng/g was partially detoxified to 86% by citric acid treatment. In addition, acidified-AFB₁ molecules from acid-treated feed, exhibit neither toxigenic activity nor carcinogenic and mutagenic activity compared with molecules of untreated feed. Additionally, AFB₁ is reported to be highly sensitive to UV irradiation at a pH of less than 3 or greater than 10 (Lillard & Lantin, 1970). Rustom (1997) mentioned that heat treatment at pH 8.0 was not effective

to reduce AFT's mutagenic activity. However, the treatment at pH 10.2 for 121°C for 15 min reduced the mutagenicity to 88%. The third possible degradation mechanism is the effect of the high temperature generated from infrared wave length. ELISA and HPLC-MS / MS results showed almost complete AFT reduction in without-skin peanuts (Table 5-1, 5-2; Figure 5-1) which was soaked in citric acids and not washed before the PL treatment. These results showed that the citric acid enhances the PL efficiency in terms of AFT destruction.

Table 5-1. ELISA results for the comparison between citric acid + PL (shaking) treatment and citric acid treatment for without-skin (w/o skin) and with-skin peanuts

Samples	Time(s)	Temperature (°C)	Reduction%
C-with skin	0	21.7 ± 1.02	0
C-w/o skin	0	21.75 ± 1.02	0
PL, w/o skin/sh	210	125 ± 10.6	73.9 ± 3.13b
PL, with skin/sh	210	130 ± 2.05	40.9 ± 14.6a
PL, with skin/sh	240	140 ± 9.19	78.9 ± 0.4bc
PL, w/o skin/sh	240	168 ± 9.19	87.1 ± 10b
Ci-with skin	3600	20.1 ± 0.0	20.6 ± 0.08a
Ci-w/o skin	3600	20.1 ± 0.0	29.3 ± 3.45a
Ci+PL-w/o skin	240	178 ± 4.7	98.1 ± 1.13c
Ci+PL-with skin	240	168 ± 2.4	88.2 ± 1.98c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD system for mean differences analysis. C = control, Ci = citric acid treatment, PL = pulsed light treatment, Ci+PL = Citric acid+ pulsed light 7 cm, sh = shaking treatment.

Table 5-2. LC-MS/MS results for the comparison between pulsed light (PL) treatment and the pulsed UV light with the citric acid treatment without washing.

Treatments	AFB ₁ R%	AFB ₂ R%
240 s PL, 7 cm + Ci	98.9a	98.1a
240 s PL, 7 cm	90.3a	95.2a
Control	0	0

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA and LSD system for mean differences analysis. C = control, Ci = citric acid treatment, PL = pulsed light treatment, R%= reduction percentage.

Table 5-3. LC-MS/MS results for the comparison between citric acid treatment and citric acid followed by PL treatment for without-skin peanuts with washing.

Treatment	AFB ₁ R%	AFB ₂ R%
Citric acid (1h)	12.6 a	51.1 a
240 s shaking PL – 7 cm + Citric acid,	91.3 b	88.9 b
Control	0 c	0 c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

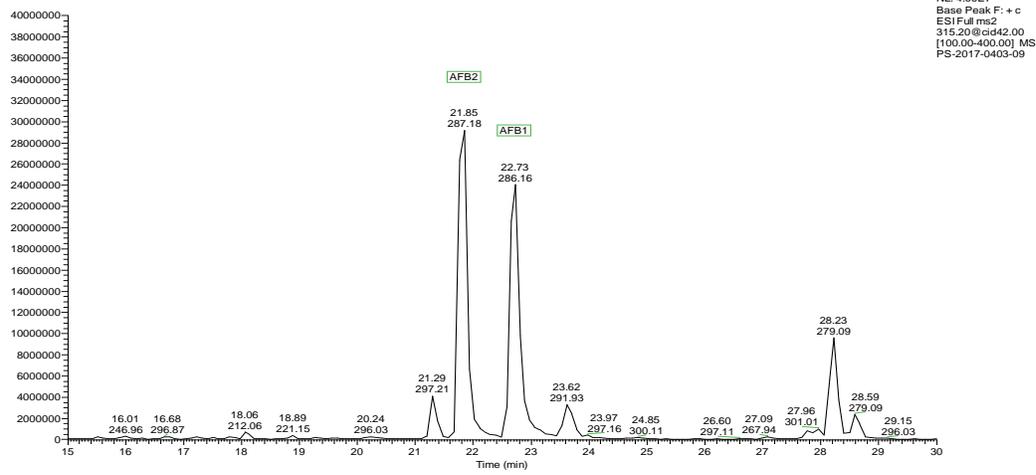
Table 5-4. LC-MS/MS results for the comparison between citric acid treatment and citric acid followed by PL treatment for with-skin peanuts with washing.

Treatment	AFB ₁ R%	AFB ₂ R%
Citric acid (1h)	19.1 a	24.2 a
240 s shaking PL – 7 cm + Citric acid,	79.1 b	80.1b
Control	0 c	0 c

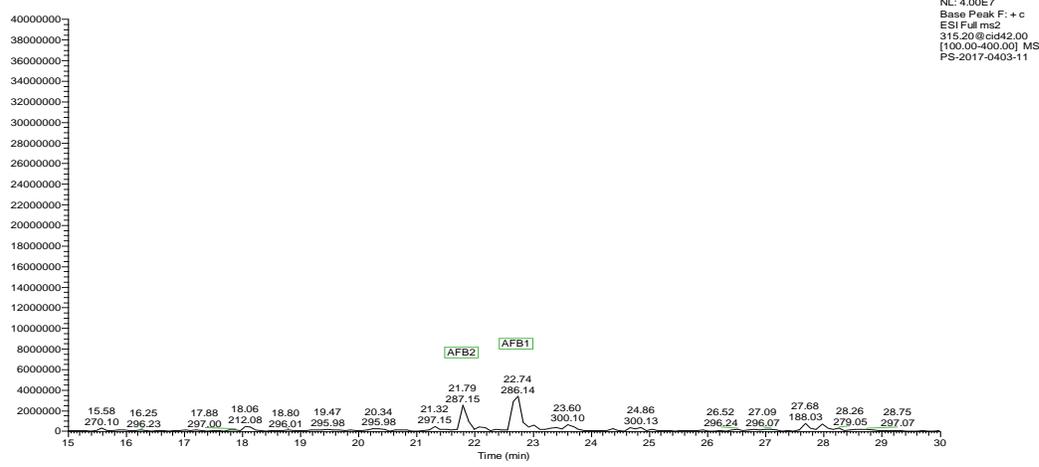
Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

A

RT: 15.00 - 30.00

**B**

RT: 15.00 - 30.00

**C**

RT: 15.00 - 30.00

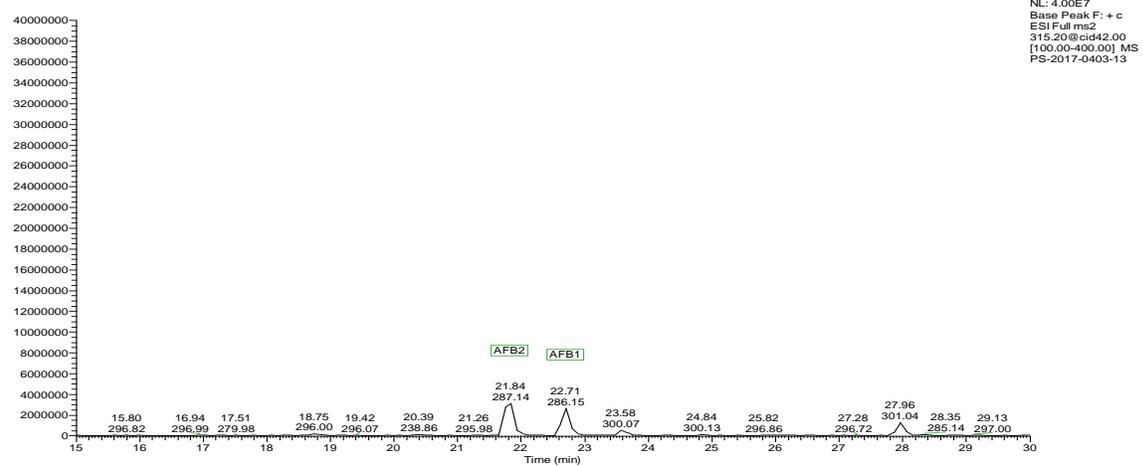


Figure 5-1. LC/MS/MS chromatograms for peanuts. A) Without treatment (control), B) After PL treatment. C) After PL + citric acid treatment.

CHAPTER 6

THE EFFECT OF STORAGE TIME ON THE PEROXIDE VALUE, FREE FATTY ACID CONTENT, AND COLOR IN PL TREATED PEANUTS

Raw peanuts and its products are important foods all over the world and are part of many famous recipes. Consumers typically use peanuts in food products in different ways (Camargo et al., 2012). Peanuts recently have been identified as contributing to lower occurrence of coronary heart disease (CHD). Peanuts contain many vitamins, and are considered an excellent vitamin E source. Peanut butter alone provides 2.3% of the vitamin E needed in the diet of the U.S. (Chun et al., 2005). Lipids are found in a great percentage in peanuts, almost 47–52% of the dry weight. The majority of the lipid composition is around 80% unsaturated fatty acids. However, greater amounts of unsaturated fatty acids accelerate rancidity, which affects the sensory quality, and flavor attributes in peanuts. Lipid oxidation is the main reason behind the off-flavor of rancid peanuts. Oxidation at later stages form the directly off-flavor contributed compounds such as alcohols, ketones, and aliphatic aldehydes. For peanuts, these chemicals are related to painty, oxidized, and cardboardy flavors connected to peanut flavor fade (Powell, 2004). Oxidation is mainly affected by peanut composition, processing method, treatment temperature, and storage conditions, including temperature, time, light and oxygen (Riveros, 2010).

Peroxide value (PV) is an indicator of the initial stages of oxidative changes. This method evaluates the total hydroperoxide content, which is one of the most common quality indicators of lipids through storage periods (Shahidi and Zhong, 2010). UV irradiation initiates free radical oxidation and forms lipid radicals, superoxide radical (SOR), and H_2O_2 , and then can lead to cross linking of carbohydrate and protein. Therefore, there are some changes in the chemical composition of food components

and possibly some product quality degradation that could occur when UV light treatment is used, especially when large fluences are applied (Kolakowska, 2002). However, from other study results, UV irradiation may not cause any components altered if UV irradiation is used in small to moderate amounts (Diao et al., 2015).

Materials and Methods

Samples Preparation and Storage Condition

Raw with-skin (with testa) and without-skin (w/o testa) peanuts were purchased from a local market. Five grams of peanuts samples were treated by Pulsed Light at 5 and 7 cm for several time periods and different methods (240 s shaking treatment, 240 s in-plate treatment, 300 s shaking treatment, and 300 s in-plate treatment). Then 5 g of each treatment was placed in small plastic bag, sealed thermally, and stored at ambient temperature inside a cardboard box to prevent excess light.

Experiment Design

Each month samples were analyzed for their peroxide value, free fatty acids, and acid value content. Factorial two-way ANOVA statistical analyses were conducted. Means and standard deviation were determined with $\alpha = 0.05$ confidence interval. Then the least significant differences (LSD) for mean differences analysis to determine the effect of stored time and different treatment on the shelf life of the PL stored peanuts was conducted.

In this experiment, the effect of PL and storage time on lipid oxidation in the peanut kernels was determined by comparing the peroxide value (PV), free fatty acids, and the acid value of the raw peanuts (control) with 1, 2, and 3 months stored peanuts (previously PL treated peanuts). Treatments assessed were the most effective PL

treatments in terms of AFT reduction: 240 s shaking–7 cm, 300 s shaking–7 cm, and 240 s in-plate–7 cm, 300 s in-plate–7 cm treatments.

Peroxide Value (PV)

The International Dairy Federation standard method 74A (International Dairy Federation, 1991, Brussels, Belgium) was used to determine PV.

Free Fatty Acids and Acid Value Determination.

FFA and acid value were determined according to the official method Ca 5a-40 of American Oil Chemists' Society (AOCS, 1990) the same as in Chapter 3.

Color Evaluation

To quantify the color changes, a machine vision system consisting of a Nikon D 200 digital camera housed inside a light box [42.5 cm (W) x 61.0 cm (L) x 78.1 cm (H)] was used (Luzuriaga et al., 1997). The camera was used to measure the color of peanut kernels with a D65 (daylight) lamp and 10° observer angle. Each image includes traditional roasted peanuts (control) and PL peanuts as measured samples. Each image was calibrated against an orange yellow color reference tile ($L=71.4$, $a=12.2$, $b=70.14$) that was obtained from the ColorChecker Classic X-Rite Company (MI, USA). The software LensEyeSK® v10.0.0 from Engineering and Cyber Solutions Inc. (Gainesville, FL, USA) was used to analyze the images.

The Hunter L, a, and b color space is organized in a cube form. All black color was surrounding the references orange and yellow cubes were erased to maintain a precise calibration without interference from the dark border. Later to obtain correct images the samples images were subjected to background corrections, and the final images were calibrated with a standards orange and yellow colors. The maximum L is 100 (white) and the minimum L is 0 (black). A positive a value denotes red, a negative a

value for green; a positive b value for yellow, and a negative b value for blue. The coordinates of three color parameters were expressed using C* (chroma), h° (hue angle), and total color difference. The color analysis was triplicated for each sample. All samples L, B, and A values were analyzed statistically using one-way ANOVA, and the LSD mean differences method.

Results and Discussion

Peroxide Value and Free Fatty Acids Tests

The PV test was used as a shelf life indicator by detecting the early stages of the autoxidation process. This study illustrated the bigger picture of the effect of PL treatment on the lipid oxidation in light-exposed peanut kernels was the tendency of the PL to accelerate lipid oxidation. Thus, in this experiment, a long storage period was conducted to monitor the levels of lipid oxidation of PL-treated peanut kernels by determining both peroxide value and FFA percentage directly after PL treatment and after each month of a three-month storage period.

According to previous studies conducted on roasted peanuts to evaluate quality, the rate of PV increasing for roasted peanuts was significantly faster than that of raw peanuts under both air and vacuum storage conditions, which indicated the low oxidative stability of roasted peanuts. Chun et al. (2005) recorded that the shelf life of dry roasted peanuts is about 2 weeks when stored at 21°C under air. However, under vacuum, the shelf-life is extended beyond 38 weeks. Evranuz (1993) reported the shelf lives of salted roasted peanuts of 28 d at 15°C, 10 d at 25°C, and 11 d at 35°C under the assumption that the products remain acceptable until the PV reaches 25 meq/kg oil. Results (Table 6-1, 6-2; Figure 6-1, 6-3) showed that the peroxide values of stored PL treated peanuts were of good quality, because the samples were below the specified

PV of 10 meq/kg for foods containing fats (AOAC, 1995) for both peanuts with-skin and peanuts without-skin, and for the distances 5,7 cm. However, the 7 cm distance's results were better after three months of storage. The highest PV value was for the 300 s in-plate treatment. For the 5-cm treatment, the PV started at zero time with 0.89 meq/kg peanut oil, and reached 12.05 and 9.89 for without-skin and with-skin peanuts, respectively, after 3 months of storage (Table 6-3, 6-5). The AOAC has declared that peanuts are considered rancid when the PV reaches 30 meq/ kg. Peroxide value increased gradually during the storage time due to the activity of enzymes such as lipooxygenase which acts as a catalyst to unsaturated fatty acid oxidation reaction to produce hydroperoxides and the secondary products, respectively (Fennema, 1996).

FFA and Acid Value

The FFA and the acid value were determined for with-skin peanuts exposed to PL using four different treatments: 240 s shaking PL, 240 s in-plate PL, 300 s in-plate, at 5 cm from the strobe. Results were analyzed using tow-way ANOVA statistical test, to determine the significant variances among treatments and storage periods at $\alpha = 0.05$. The P-value of between treatments of FFA and the acid value was 0.42 (> 0.05). Thus, this analysis didn't show significant differences among treatments. However, there were significant differences among samples FFA and acids value before and after storage. Means comparison test, Fisher's the Least Significant Difference (LSD), method showed no significant differences at the first two months neither in the control nor in the 240 s, 300 s in-plate samples with FFA ranging from 0.43 to 0.57. However, after storage for three months, the LSD results showed that all treatments are significantly different. The least FFA for 3 months of storage were found in the 240 s PL shaking treatment (Table 6-5). The acid value had no significant differences among treatments

in the zero time and after 1-month storage, and all samples' mean were statically different at $\alpha = 0.05$ at the second and the third months (Table 6-2, 6-8).

Results of FFA (Table 6-10) show the FFA percentages and the acid value of with-skin peanuts exposed to PL using four different treatments: 240 s shaking PL, 240 s in-plate PL, 300 s in-plate, at 7 cm from the strobe. Results were analyzed using two-way ANOVA statistical test, to determine the significant differences among treatments and storage periods at confidence intervals $\alpha = 0.05$. The P-value of between treatments was 0.27 ($>\alpha = 0.05$) for both FFA and acid value. Thus, this analysis didn't show significant differences among treatments.

FFA and the acid value percentages was evaluated for without-skin peanuts samples, which exposed to PL using four different treatments: 240 s shaking PL, 240 s in-plate PL, 300 s in-plate, at 5 cm then 7 cm from the xenon lamp. Results were statistically analyzed using tow-way ANOVA test, to determine the significant differences among treatments and storage periods at confidence intervals $\alpha = 0.05$. The p-value of between treatments was 0.002, ($< \alpha = 0.05$) and 0.29 ($>\alpha = 0.05$) for 5 cm and 7 cm, respectively. Thus, the distances were a crucial factor in all treatment since the results were significantly different among treatments at 5 cm but not at 7 cm from the strobe. On the other hand, storage time effect the FFA and acid value measurements. The P-value of the storage time was 0.0002 and 0.047 ($<\alpha = 0.05$) for 5 cm and 7 cm, respectively, which means there were significant differences among samples' FFA and acid values before and after storage. The means comparison, Fisher the Least Significant Differences (LSD), test analysis showed no significant differences at the first two months between treatments. However, it showed differences among all

the storage periods times. Especially after three-month storage, all treated samples were varied in their FFA and acid values. The least FFA and acid value content was a result of 240 shaking PL treatment again for with-skin peanuts (Table 6-5, 6-6; Table 6-9, 6-10). To summarize the results, treatments at 7 cm had lower FFA than the 5-cm distance from the Xenon lamp, and treatment without-skin had more homogeneity in their results. The rate of FFA increase for PL treated peanuts was significantly faster than that of raw peanuts under the same conditions. Chun (2005) stated that peanuts treated with high-temperature such as roasting showed high susceptibility to oxidation due to the high content of unsaturated fatty acids. UV light has been found to have the ability to stimulate specific enzymes, which are responsible for the biosynthesis of the flavonoids, these enzymes may produce compounds that act as UV screens preventing UV-induced damage in the genetic material of plant cells (Cantos et al., 2000).

To compare the presented study results with what had published before, results emphasized that the maximum FFA measurement (3.26% FFA), which was recorded during this study for without-skin peanuts' sample placed in-plate at 5 cm from the strobe and treated for 300 s, and after a 3-month storage period. These results were much lower than what Makeri (2011) had from roasting peanuts using a conventional air-oven at temperature of 80, 100, 120, 140, and 160°C for 20 min and stored for 0, 3, 6, 9, and 12 weeks. Makeri (2011) FFA values ranged from 0.45 to 1.80 at time zero and from 0.90 to 2.20 after storage for 3 weeks, 6.30 to 8.20 after storage for 6 weeks, 7.80 to 9.10 after storage of 9 weeks, and 8.10 to 9.55 after the 12-week storage period. The FFA for the control untreated peanuts samples ranged between 0.40 and 0.49, which agreed with other references. Pokorny et al. (2003) recorded FFA for raw peanuts

between 0.4 and 0.72 and acid value between 0.79-1.48, and Makeri (2011) found that the FFA for raw peanuts was 0.45.

Moreover, peroxide value and FFA percentage and acid value results correlated. The peroxide value increased as the storage time increasing as well as the FFA and the acid value. With both methods, the shaking treatment for 240 s had the lowest oxidation values. Especially for the with-skin samples, all PV and FFA were lower than the without-skin, which could be due to the composition of the skin which included some polyphenols and antioxidant such as tocopherols compounds which may helped to delay the auto-oxidation of the lipids (Sales & Resurreccion, 2010). Although, Sobolev & Cole (2004) stated that regarding the general differences between with-skin and without-skin peanut, non-significant differences were found in relation to the general composition of the peanut samples ($p < 0.05$). Chun (2005) has demonstrated that tocopherol losses were highly correlated with lipid oxidation based on PV and the conjugated diene values (CDV) for all stored peanuts, indicating the antioxidant function of vitamin E during lipid oxidation.

Similarly, the results in the present study showed that the existence of the skin could have a quite significant effect on the PV and the FFA and acid value to extend the storage time (Table 6-5, 6-6, 6-7, 6-8).

Color Evaluation

Zhao (2013) mentioned that some parts of peanut samples that were treated with PL had darker spots if the peanut kernel was not spun. However, using the shaking treatment succeeded to reduce AFTs without any loss in the color quality of the peanuts. In this study L-value, b-value, and a-value of three different PL treatments (180 s / 7 cm, 210 s / 7 cm, and 240 s / 7 cm) were compared with commercial (Publix

roasted peanuts) and a control (non-treated peanuts) to find out the effect of PL treatment on the peanuts degree of roasting and the uniformity of color. All samples were evaluated using machine vision. Samples *L, *a, *b levels revealed that all samples tend to give greyish yellow color, since the *a value gives negative numbers and the *b value was low positive number (Table 6-13). Typically, roast color is the most important quality control parameter in commercial processes. Roast color in peanuts is generally measured by light reflectance in a colorimeter, giving an L-value in a range from 80 (very light or no roast) to 30 (very dark roasted). The Hunter L-value of roasted peanuts used in high quality dry roasted peanuts and peanut butter falls in the range of 50–51 (Sanders et al., 1989; Baker et al., 2003). In the present study the L-value, which described the whitening of the tested samples ranged from 70.86 for the commercial roasted samples to 76.38 for control peanuts (Table 6-13). A one-way ANOVA statistical analysis followed by LSD means differences analysis revealed that neither between samples nor within each sample had significant differences at $\alpha = 0.05$ except the commercial sample in its *a level (describe the red intensity of the red color), which was significantly higher than the *a level for the control and all other PL treated samples. The *b Value described the greenish yellow of the samples revealed that the commercial sample wasn't significantly different from other samples. However, it was much higher than all the PL treated sample, and the control with the b-value ranged from 24.5 to 25.21. Measurements of the same samples showed a negative a-value ranged from -3.61 to -5.07 for the PL treated samples and ranged from -5.37 to -5.53 for the control non-treated peanuts. However, for commercial sample ranged from -1.33 to -1.61. All other samples had no significant differences in their Chroma and ΔE values

with the control and the different PL treatments. Which clarified that the color of the PL treated samples until the treatment of 240 s was uniform and it wasn't significantly different from the control (Table 6-14).

Color evaluation results showed no significant differences between the three different treatments and the control, but there were significant differences between the treated samples and the commercial sample. The level of roasting after all the 180 s, 210 s, and 240 s PL treatments fell into the roast category of light. Means comparison by LSD analysis showed no significant differences at $\alpha = 0.05$ neither between samples nor within samples for color (Table 6-13; Figure 6-1). The shaking treatment provided good uniformity and resulted in peanuts with a homogeneous color.

Table 6-1. The peroxide value for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 5 cm distance from the strobe.

Time (month)	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
0	2.1a	1.67a	2.46a,b	1.33a	2.77a,b
1	3.13a	1.57a	2.23a,b	1.1a	3.1a,b
2	3a	3.33a,b	3.53b	4.33a,b	6.67b
3	2.03a	6.03b	6.9b	6.6b	9.89c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-2. The peroxide value for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 7 cm distance from the strobe.

Time (month)	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
0	1.1a	1.63a	1.91a,b	1.76a	2.17a,b
1	1.13a	1.23a	2.17a,b	1.5a	2.77a,b
2	2a,b	2.86a,b	3.4a,b	3.26a,b	4.66b
3	2.03a,b	4.73b	6.67b,c	6.67b,c	7.7c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-3. The peroxide value for 0, 1, 2, and 3 months stored PL treated without-skin peanuts at 5 cm distance from the strobe.

Time (month)	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
0	2.8a	2.67a	2.96a	2.83a	2.77a
1	3.03a	2.57a	2.23a	3.1a,b	3.1a
2	3.05a	6.78a	8.53a,b	5.33a,b	8.86a,b
3	4.13a	8.13a,b	10.9a,b	7.6a,b	12.89b

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-4. The peroxide value for 0, 1, 2, and 3 months stored PL treated without-skin peanuts at 7 cm distance from the strobe.

Time (month)	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
0	2.8a	2.67a	2.96a	2.83a	2.77a
1	2.13a	2.23a	2.97a	1.5a	3.77a,b
2	3a	4.86a,b	5.4b	3.26a,b	6.66b,c
3	3.13a	6.73b,c	7.67b,c	7.69b,c	9.74c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-5. The FFA % for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 5 cm distance from the strobe.

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.45a	0.43a	0.51a	0.45a	0.46a
Month 1	0.54a,a	0.57a	0.57a	0.56a	0.53a
Month 2	0.69a	0.65a	0.78b	0.61a	0.78b
Month 3	0.61a	1b	1.34c	2.01d	2.87e

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-6. The FFA % for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 7 cm distance from the strobe.

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.43a	0.43 a	0.51a	0.49a	0.49a
Month 1	0.58a	0.59 a	0.63a	0.6 a	0.61a
Month 2	0.59a	0.73 b	0.78b	0.69a	0.93b
Month 3	0.6a	1.05c	1.17c	2.23d	2d

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-7. The FFA % for 0, 1, 2, and 3 months stored PL treated w/o skin peanuts at 5 cm distance from the strobe.

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.49a	0.54a	0.59a	0.59a	0.7a
Month 1	0.53a	0.75a	0.76a	1.5a,b	1.77b,c
Month 2	0.64a	0.56a	0.78a	1.04a,b	2.02c
Month 3	1.02a,b	1.68c	1.98c	2 c	3.26d

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-8. The FFA % for 0, 1, 2, and 3 months stored PL treated w/o skin peanuts at 7 cm distance from the strobe.

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.44a	0.54a	0.65a	0.65a	0.59a
Month 1	0.51a	0.54a	0.72a	0.63a	0.79a
Month 2	0.56a	0.59a	2.53c	0.65a	1.94 b
Month 3	0.60a	1.58b	1.88b	1.87b	2.45c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-9. The acid value results for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 5 cm distance from the strobe

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.90a	0.86a	1.02a	0.90a	0.92a
Month 1	1.08a	1.13a	1.13a	1.11a	1.06a
Month 2	1.37a	1.29a	1.55b	1.21a	1.55b
Month 3	1.21a	1.99 b	2.67c	4.00d	5.71e

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-10. The acid value results for 0, 1, 2, and 3 months stored PL treated with-skin peanuts at 7 cm distance from the strobe

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.86a	0.86a	1.02a	0.98a	0.98a
Month 1	1.15a	1.17a	1.25a	1.19a	1.21a
Month 2	1.17a	1.45a,b	1.55a,b	1.37a	1.85b
Month 3	1.19a	2.09 b	2.33 b	4.44c	3.98d

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-11. The acid value results for 0, 1, 2, and 3 months stored PL treated w/o skin peanuts at 5 cm distance from the strobe

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.98 a	1.08 a	1.17a	1.17a	1.39a
Month 1	1.06 a	1.49 a	1.51a	2.99b	3.52c,d
Month 2	1.27 a	1.11 a	1.55a,b	2.07b	4.02d
Month 3	2.03 a,b	3.34 c	3.94d	3.98 d	6.49e

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA

Table 6-12. The acid value results for 0, 1, 2, and 3 months stored PL treated w/o skin peanuts at 7 cm distance from the strobe

Storage time	Control	240 s shaking	240 s in-plate	300 s shaking	300 s in-plate
Month 0	0.88a	1.08a	1.29a	1.29a	1.17a
Month 1	1.05a	1.08a	1.43a	1.25a	1.57a
Month 2	1.11a	1.17a	7.02d	1.29a	3.86b
Month 3	1.19a	3.14b	3.74b	3.72b	4.88c

Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-13. L*, a*, b* values for color evaluation of commercial peanuts roasted peanut, control (untreated peanut), and four different times PL treated peanut samples.

	Commercial	0 s	180 s	210 s	240 s
L* value	70.86 ± 6.21a	74.33 ± 4.49a	73.85 ± 4.76a	75.23 ± 3.96a	74.1 ± 4.76a
	73.49 ± 6.38a	76.38 ± 3.93a	75 ± 4.53a	75.49 ± 4.95a	76.79 ± 4.22a
	71.87 ± 5.65a	75.11 ± 4.93a	75.12 ± 4.6a	76.31 ± 3.85a	74.39 ± 4.72a
b* value	24.5 ± 6.22b	18.56 ± 5.31b	18.5 ± 4.38b	19.6 ± 4.49b	19.94 ± 4.5b
	25.95 ± 6.79b	20.21 ± 4.22b	19.79 ± 4.32b	20.98 ± 5.57b	19.2 ± 3.99b
	25.21 ± 5.88b	19.01 ± 4.02b	19.07 ± 4.1b	17.88 ± 3.77b	19.74 ± 3.83b
a* value	-1.61 ± 3.84c	-5.53 ± 1.66d	-5.07 ± 2.04d	-4.87 ± 1.9d	-4.05 ± 2.37d
	-1.32 ± 4.02c	-5.37 ± 1.53d	-4.66 ± 2.03d	-3.52 ± 2.8c,d	-4.35 ± 1.77d
	-1.33 ± 3.39c	-5.44 ± 2.28d	-4.85 ± 1.89d	-4.88 ± 1.62d	-3.61 ± 2.36d

*Average of three samples within the same sample. Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

Table 6-14. Hue angle, Chroma, and whiteness values for color evaluation of commercial roasted peanut, control (untreated peanut), and PL treated peanuts.

	Commercial	0 s	180 s	210 s	240 s
Hue Angle	-73.5 ± 145a	-139 ± 39.9b	-134 ± 59.3b	-142 ± 47.8b	-127 ± 87.6b
	-63.3 ± 151a	-144 ± 37b	-138 ± 62.9b	-113 ± 107b	-141 ± 57.4b
	-66.5 ± 151a	-136 ± 52.7b	-141 ± 48.5b	-142 ± 41.4b	-134 ± 80.8b
Chroma	24.9 ± 6.07a	19.5 ± 4.95a	19.4 ± 3.96a	20.4 ± 4.05a	20.6 ± 4.13a
	26.3 ± 6.66a	21.0 ± 3.9a	20.5 ± 3.94a	21.6 ± 4.18a	19.8 ± 3.63a
	25.5 ± 5.69a	20.0 ± 3.61a	19.8 ± 3.75a	18.7 ± 3.42a	20.3 ± 3.56a
ΔE	72.7 ± 5.14a	76.0 ± 5.43a	75.4 ± 3.59a	75.0 ± 6.49a	74.6 ± 5.67a

*Average of three samples within the same sample. Different alphabetic means significantly different at $\alpha = 0.05$ using one-way ANOVA.

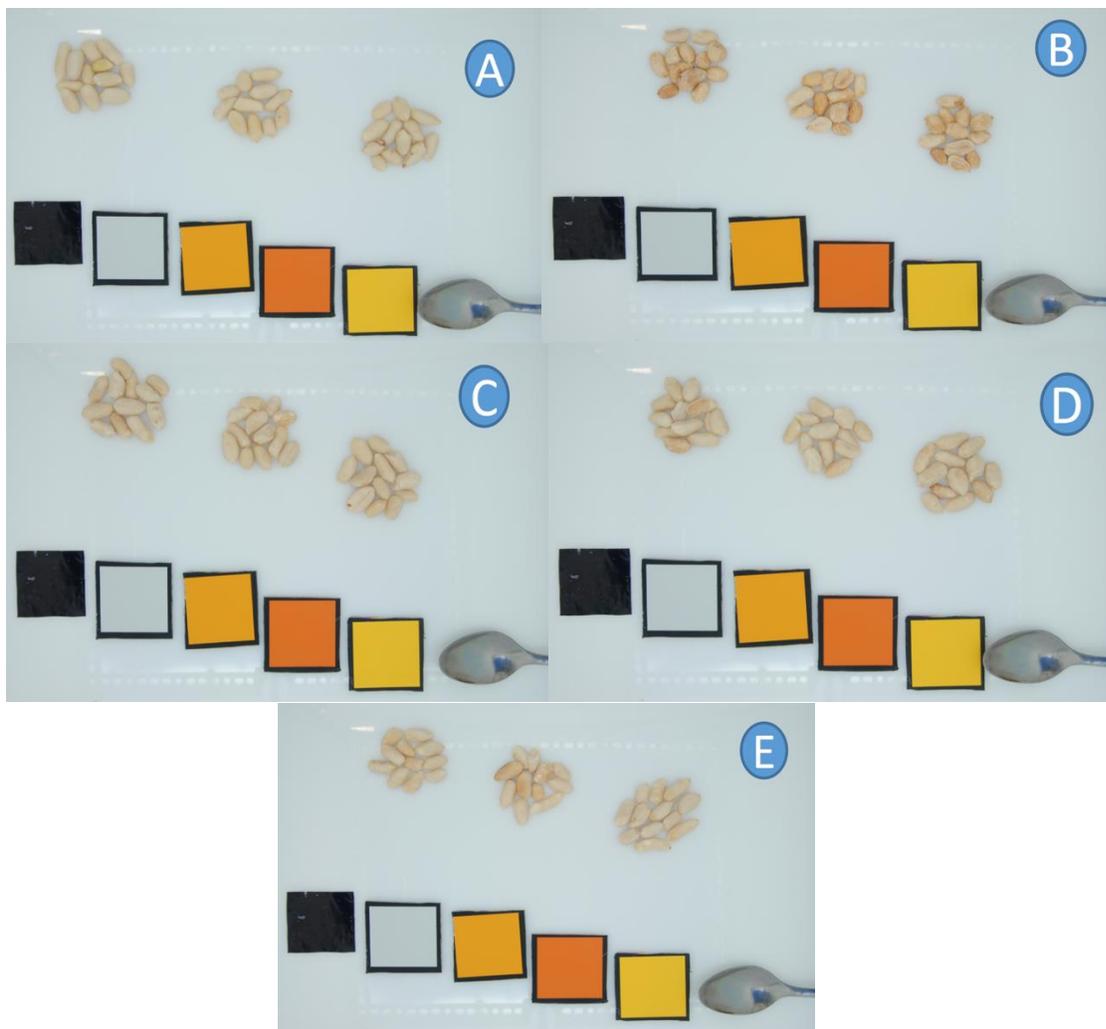


Figure 6-1. The color vision machine images for peanuts samples. A) Control (untreated peanuts), B) Publix Commercial roasted peanuts C) 180 s per 7 cm PL treatment D) 240 s per 7 cm PL treatment E) 210 s per 7 cm PL treatment (Photo courtesy of author).

CHAPTER 7 OVERALL CONCLUSION

Pulsed light (PL) has been considered a non-thermal technique and has been used successfully to eliminate bacteria and viruses using very short exposure times. Short duration of PL treatment didn't cause an elevation in food temperature. However, in this study since the AFTs are extremely stable compounds, especially in solid food such as peanuts, relatively long exposing times were used to reach a sufficient level of AFT degradation. PL treatment achieved complete AFT reduction when treated in solvent. Overall, this study suggested that there is a good potential to optimize peanut quality by simply adjusting the time/temperature profiles during PL treatment. Treatments for ≥ 240 s at 5 cm or 7 cm almost achieved complete AFT detoxification. Among the five different treatments which were investigated in this study, the shaking treatment was the best treatment in terms of uniformity of the treatment which led to higher AFB₁, AFB₂ reduction along with homogeneous color distribution.

PL irradiation is a successful technique in terms of AFT degradation. However, all other parameters, especially the moisture content, have a robust influence on AFT degradation. With PL technique, samples should be placed accurately right under the quartz window of the Xenon lamp. The sensitivity of the without-skin samples to fungi contamination, PL treatment, and experiment parameters changing was higher than the with-skin samples.

The hurdle technique by adding citric acid treatment enhanced the PL treatment, and the AFTs were dramatically reduced in most treatments, especially after 240 s of PL treatment. Peroxide value, FFA, and acid value for PL treated samples revealed that PL treatment didn't have a detrimental effect on peanut quality even after 3 months of

storage. However, UV light has been known for its ability to accelerate the lipid oxidation; thus, the low peroxide value could be due to the fast production of the final product of the unsaturated fatty acids oxidation. Therefore, for future work, gas chromatography analysis should be conducted to profile the secondary oxidation products originating from the oxidized lipids.

APPENDIX A
INOCULATION OF WITHOUT-SKIN AND WITH-SKIN PEANUTS WITH
ASPERGILLUS FLAVUS

A. flavus strains should be prepared by growing on dextrose agar for 5-7 d with



After incubation at 30°C, spores will be harvested then filtered using cheesecloth, then counted using Hemocytometer.



500 g of peanuts will be placed in Erlenmeyer flasks (150 mL), 50 mL of distilled water will be added and autoclaved at 121°C for 1 h and will be allowed to stand overnight.



Each flask will be inoculated with 25 mL of the spore suspension, incubated at $29 \pm 1^\circ\text{C}$ in a dark place for 21 d and shaken once or twice daily.

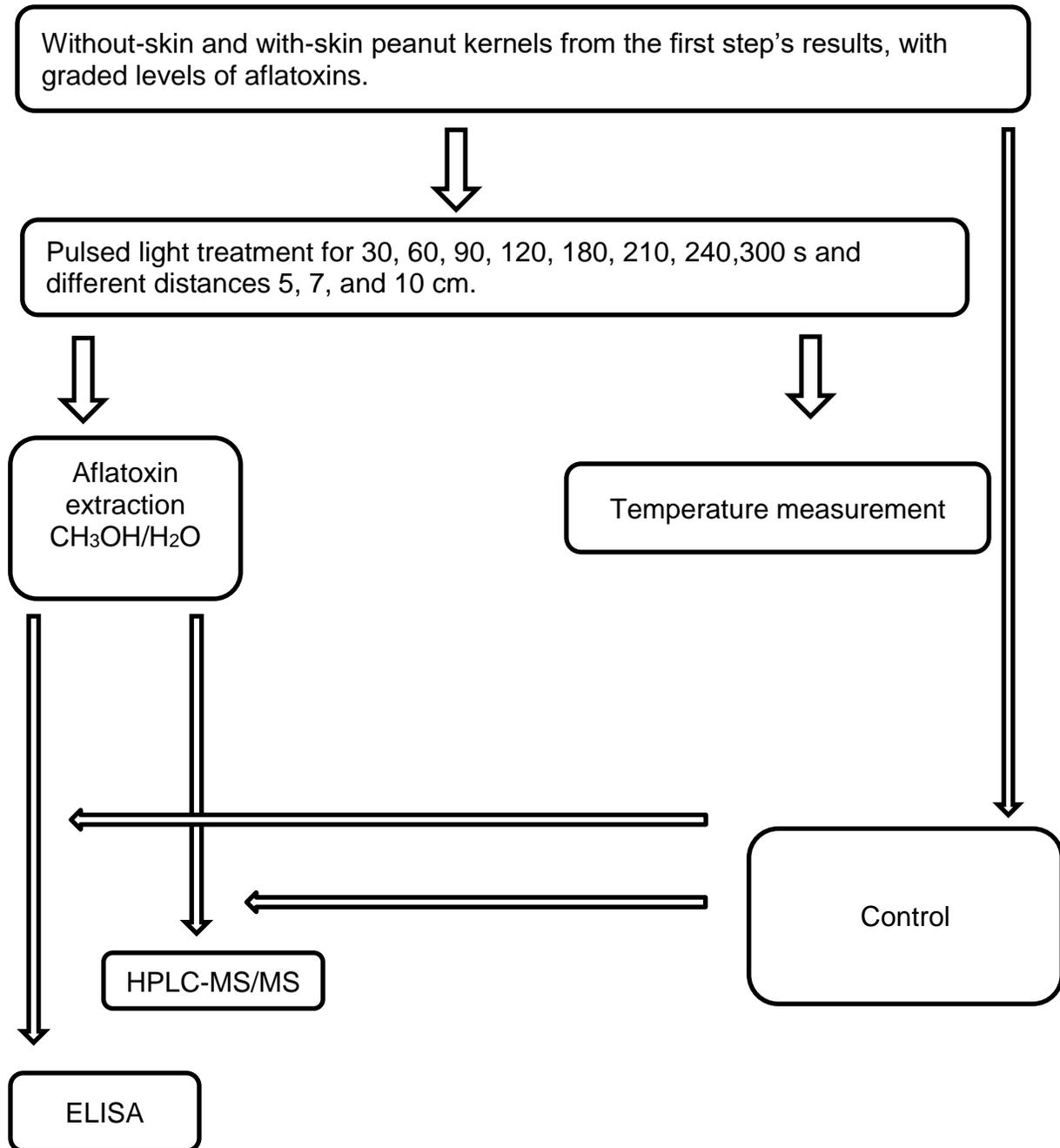


Aflatoxin extraction $\text{CH}_3\text{OH}/\text{H}_2\text{O}$



Aflatoxin determination by ELISA and HPLC-MS/MS

APPENDIX B
PULSED LIGHT TREATMENT



APPENDIX C
COMBINE PULSED LIGHT WITH CITRIC ACID AS A HURDLE TECHNIQUE AND
COMPARE THE RESULTS WITH THE PL TREATMENT

Without-skin and with-skin peanut kernels from the first objective's results,
with different levels of aflatoxins.



The previously contaminated peanuts will be treated with 0.1 N aqueous
citric acid for 1 h (2 mL per gram of contaminated peanuts).



Pulsed UV light treatment for 210 and 240,300s shaking treatment and
at 7 cm.



Aflatoxin extraction: CH₃OH/H₂O

ELISA

HPLC-
MS/MS

Result comparison for objectives two and three: Statistical
analysis.

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BIOGRAPHICAL SKETCH

Manal Othman Abuagela was born in Tripoli, Libya. She received her bachelor's and master's degrees from Department of Food Science, College of Agriculture, University of Tripoli (Al-Fateh University) in 1993 and 2006, respectively. After her bachelor's degree, she was a teacher for different school levels from elementary to high school. Generally, teaching Chemistry. She started her master's studies at the University of Tripoli in 2000. After her master's degree, she worked as an assistant professor at the University of Souk-Al-Ahad. Al-Zaytoonah University (Nasser) since 2006–2013. She started her Ph.D. studies at Department of Food Science and Human Nutrition at the University of Florida in 2013 under Dr. Wade Yang. She then transferred to be under Dr. Paul Sarnoski.