DETOXIFICATION OF AFLATOXIN IN PEANUT MEAL BY HEATING AND GAMMA IRRADIATION

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ŞÜKRAN GİZEM AYGÜN

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DETOXIFICATION OF AFLATOXIN B1 IN PEANUT MEAL BY ROASTING AND GAMMA IRRADIATION

submitted by **ŞÜKRAN GİZEM AYGÜN** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Gülbin Dural Ünver _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Alev Bayındırlı _____
Head of Department, **Food Engineering Dept., METU**

Prof. Dr. Faruk Bozoğlu _____
Supervisor, **Food Engineering Dept., METU**

Assoc. Prof. Dr. İlkay Şensoy _____
Co- Supervisor, **Food Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Alev Bayındırlı _____
Food Engineering Dept., METU

Prof. Dr. Faruk Bozoğlu _____
Food Engineering Dept., METU

Prof. Dr. Mehmet Mutlu _____
Biomedical Engineering, TOBB University

Assoc. Prof. Dr. Deniz Çekmecelioğlu _____
Food Engineering Dept., METU

Assoc. Prof. Dr. Özlem Tokuşoğlu _____
Food Engineering Dept., Celal Bayar University

Date: 01.06.2015

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Şükran Gizem, Aygün

Signature:

ABSTRACT

DETOXIFICATION OF AFLATOXIN B1 IN PEANUT MEAL BY HEATING AND GAMMA IRRADIATION

Aygün, Şükran Gizem

Ph.D., Department of Food Engineering

Supervisor: Prof. Dr. Faruk Bozoğlu

Co-Advisor: Assoc. Prof. Dr. İlkay Şensoy

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Aflatoxin is one type of mycotoxin, which is synthesized by some molds as by-products under certain conditions and needed to be controlled because of several health risks when present in foods and feeds. Due to these health related effects, prevention of aflatoxin in food and feed is essential.

According to the Turkish Food Codex Communiqué on Undesirable Substances in Feed, the maximum limit of aflatoxin B1 in feed materials is defined as 0.02 ppm. In case of unavoidable aflatoxin contamination, there are variety of techniques for detoxification of aflatoxins such as fermentation, extraction, extrusion, solar treatment, oxidizing agents and gamma irradiation.

In the scope of this thesis, naturally aflatoxin contaminated peanut meal were subjected to different treatments (roasting (120°C, 160°C and 200°C) and gamma

irradiation (10 kGy)) in order to reduce Aflatoxin B1 content to the levels specified by Food Codex. Studies on residual toxicity after treatments were also carried out to test whether the toxicity increases due to by products formed upon treatments.

According to the results obtained from our experiments; high temperature roasting (200°C) and gamma irradiation (10 kGy) were more effective on aflatoxin B1 reduction (~80%). Roasting especially at high temperatures (200°C) and gamma irradiation reduced the level of protein content of peanut meal samples. Minimum reduction in the protein content was observed in samples treated with gamma irradiation. In addition to the reduction levels, used techniques did not have an increasing effect on toxicity of Aflatoxin B1.

Keywords: *Aflatoxin B1,Detoxification, Peanut, Roasting, Gamma Irradiation*

ÖZ

YERFISTIĞI YEMLERİNDE KAVURMA VE GAMA IŞINLAMA İLE AFLATOKSİN B1'İN DETOKSİFİKASYONU

Aygün, Şükran Gizem
Doktora, Gıda Mühendisliği Bölümü
Tez Yöneticisi: Prof. Dr. Faruk Bozoğlu
Ortak tez Yöneticisi: Yrd. Doç. Dr. İlkay Şensoy

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Aflatoksin küfler tarafından belirli koşullar altında yan ürün olarak üretilen ve gıda ve yemlerde bulunması durumunda gerçekleşen birçok sağlık riski nedeniyle kontrol edilmesi gereken bir mikotoksin çeşididir. Bahsi geçen sağlık bağlantılı etkileri nedeniyle; gıda ve yem maddelerinde aflatoksin oluşumunun engellenmesi esastır.

Türk Gıda Kodeksi Yem Maddelerinde İstenmeyen Maddeler Tebliği'nde, yem maddelerinde Aflatoksin B1'in en yüksek limiti 0.02 ppm olarak belirtilmiştir. Önlenemeyen aflatoksin bulaşması durumunda; fermentasyon, ekstraksiyon, ekstrüzyon, güneş ışını uygulaması, oksidasyon ajanları ve gama ışınlaması gibi çeşitli teknikler aflatoksindetoksifikasyonu için uygulanabilmektedir.

Bu Tez kapsamında; doğal yollarla aflatoksinlekontamine olmuş yerfıstığı numuneleri, aflatoksin düzeyinin Gıda Kodeksinde belirlenen seviyelere düşürülebilmesi için farklı uygulamalara (kavurma (120°C, 160°C, 200°C) ve gama

ışınlaması (10 kGy)) tabi tutulmuştur. Uygulamalar sonrası oluşan yan ürünler sonucu toksisitenin artma olasılığının anlaşılabilmesi için kalan toksisite üzerinde çalışmalar yürütülmüştür.

Deneyler sonucunda elde edilen sonuçlar, kavurmanın (200°C) ve gama ışınlamasının (10kGy) aflatoksin B1 miktarının azalması üzerinde daha etkili olduğunu göstermiştir (~%80). Kavurma özellikle 200°C'de ve gama ışınlaması, yerfıtığı yem numunelerinin protein içeriği üzerinde azalmaya sebep olmuşlardır. En düşük seviyede protein miktarı azalması gama ışınlamasında gözlenmektedir. Aflatoksin B1 azalma miktarlarına ek olarak, uygulanan teknikler, aflatoksinin toksik etkisi üzerinde artışa sebep olmamıştır.

Anahtar Kelimeler: *Aflatoksin B1,Detoksifikasyon, Yerfıstığı, Kavurma, Gama Işınlaması*

To my mother, my love and baby

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TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Animal feeding has an important status in the global industry, which makes the economic production of animal protein worldwide possible. For providing safe and economical animal protein source; feed is the most important component. Worlds annually feed production is nearly 1 billion tones. One of the most important challenges in feed industry is the amount of traditional production of feed, it is nearly 300 million tons of uncontrolled traditional mixing production on farms through 1 billion tons of feed production totally. This makes the feed materials hard to control by regulatory authorities. It is vital to control whole feed chain by authorities with clear standards to obtain sustainability of food and feed chain (International Feed Industry Federation, 2010).

1.1 Feed

Animal feed is for the domestic animal food used in animal husbandry. Feed production needs not only careful management of raw material quality but also production steps. Generally feed contains cereals, cereal by products and proteins and also minerals, vitamins and feed additives as co- products(Feed Milling, 2013).

It is essential that the nutritional value of the raw materials is retained by preventing deterioration of the feed once it has been manufactured.

One of the most common animal feed in animal husbandry is peanut originated feed (peanut meal) (Figure 1.1). Peanut is originated from legume family and its fruits grow under the ground which brings the difference of peanut from the other cereal crops (TEAE, 2007). Peanut is a rich source of vitamin B and contains 25% protein, 46% oil, 16% carbohydrate and 5% mineral. Its fruits contain phosphorus rich amino acids. 100 gr of peanut has 600 kcal of energy and its oil content changes from 40-60%, in general. Peanut meal is produced from whole or broken peanuts by removal of peanut oil by extraction (Batal, et.al, 2005). In contrast to soybean meal, peanut meal is low in lysine but is an excellent source of arginine, phenylalanine and cysteine which makes its protein content higher.

Figure 1. 1 Peanut Meal as Animal Feed

As well as being food for human consumption, peanut is used in large quantities, in several areas of the industry. Peanut oil is used as cooking oil in solid and liquid form, used for fish canning, biscuits, cakes, and candies as well as, used in soap production. Cake that remains after the removal of oil is a valuable feedstuff. Due to the fact that; peanut is a legume plant, it is a very valuable material as animal feed.

1.1.1 Feed Market and Regulations in Turkey

The feed industry is growing very fast and growth will continue to increase steadily in the coming years. There are a lot of new investments in Turkey for both dairy and recently in cattle feed lots (USDA GAIN: Turkey Grain and Feed Annual 2013).
Since 2004, peanut cultivation has gained importance in some regions of Turkey. 98.81% of the peanut cultivation in Turkey has been carried out in Osmaniye, Adana, Mersin, Aydın, Muğla and Antalya. In World's production, share of Turkey is very low. In 2004; Turkey had peanut production of 80,000 tons and in 2014, the production has reached to 123,600 tons. By this rate; Turkey only has 1% of the total World production rate (TUİK, 2015).

Peanut as a feed material is excellent substrate for mold and yeast growth as well as, mycotoxin production. Aflatoxin is the most important cause of economic and product losses with high management control costs among all mycotoxins because of its high toxicity and long history of regulations. Aflatoxin problem is not only dangerous for human health but also important in the view of economic loss. The reports published by FAO also remark the excess of economic losses due to mycotoxin contamination in the world. These economic losses increase the product loss of producer, loss of animal and milk, and negatively affect the distributors by rising cost, rising prices and health expenses. Because of all these reasons, prevention of Aflatoxin in food and feed is essential.

Technical and hygienic requirements to ensure the control of feed production and quality, GMO traceability and requirements for medicated feed production are set by the Law No. 5996 on Veterinary Services, Plant Health, Food and Feed prepared by the Ministry of Food Agriculture and Livestock of Republic of Turkey.

The Law identifies the feed as all types of materials and products, including all processed, semi-processed or unprocessed materials and additives for the oral feeding of animals.

In addition, the same Law identifies the requirements for the feed safety as follows:

a) Unreliable feed products shall not be put upon the market or be used for feeding the animals
b) Feed product is accepted as unreliable in case of causing negative effects to human and animal health or causing untrusting situation for the human consumption of food products obtained from animals, according to intended use.
c) Spoiled or rotten feed is evaluated as improper for the consumption.
d) When a serial, batch or shipment of a portion of feed from the same class or kind is referred as unreliable, as a result of the assessment; comprehensively; the rest should not be proven to be reliable, meaning the serial, batch or shipment of all varieties from the same class are deemed to be untrusted.
e) Even though, the feed complies with the conditions specified by the Ministry, in the case of doubt at sufficient amount or reason for the feed to be unreliable, the Ministry can restrict placing or marketing of the mentioned feed.

1.1.2 Production of Peanut Meal

Peanut (*Arachishypogaea L.)* is fourth important oilseed crop grown all over the world. China and India lead in peanut production followed by Nigeria, U.S.A and Indonesia. Peanut has gained significance in the recent years as a protein source due to its high protein content (Yadav et.al, 2013).

Peanuts are important crops in Southeast as human food and feed for livestock. The most valuable by- products from crushing peanuts for oil is the peanut cake or meal. It is a valuable feed for all classes of livestock and poultry because of its high protein content. Peanut meal is mostly used by mixed feed manufacturers due to its stable content (Sheely, et.al, 1942)

Peanut meals are generally produced by traditional methods. The first step of production starts with the crushing of kernels consists of polyphenolic compounds

and also germs with bitter aroma, and known amount of hulls for improving extraction efficiency. At the end of this process, fiber rich, dark in color and bitter in taste product is obtained. In order to be a rich source of protein, this products need to be suitably processed to remove the undesirable compounds. The intermediate products obtained after crushing is extracted with n-hexane and 80% ethanol for 2 hours. At the end of the extraction; product is directed to the expeller press and peanut meal is obtained after pressing and removal of solvent (Smith, 1972). A brief figure explaining the oil seed extraction procedure is given in Figure 1.2.

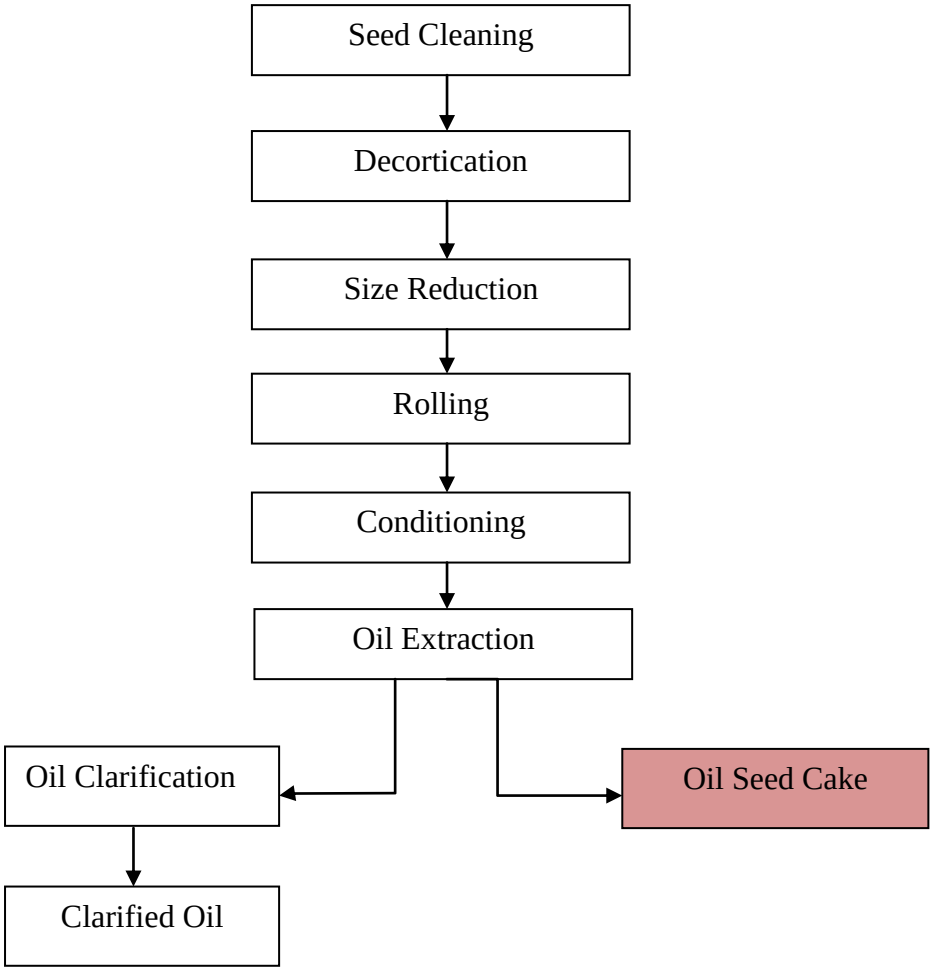


Figure 1.2 Extraction of Oil from Crude Peanut Meal (Head, et.al, 1995)

1.2 Aflatoxin

The first sight of aflatoxin was in 1960 in an English poultry farm with more than 100,000 young turkeys. Within few months, there is a huge mortality rate of this disease and this new disease was called as "Turkey X disease". It was soon found that the disease was not limited to turkeys, but the diseases all associated with feeds, Brazilian peanut meal. Studies on peanut meal were conducted and the results revealed that the meal has toxic components to poultry and ducklings like in the case of Turkey X disease. The nature of the toxin suggested that it might be of fungal origin. In 1961, *Aspergillus flavus*, a type of fungus, was identified as the toxin producer and according to the virtue of its origin, toxin was named as Aflatoxin (Eaton & Groopman, 1994). According to this survey; Aflatoxins (Figure 1.3) the most widespread type of mycotoxin known ever, are produced by *Aspergillus flavus, Aspergillus paraciticus, Aspergillus nomius* and also some *Penicillium* and *Rhizopus* species. Aflatoxins are metabolites that cause acute and chronic poisoning in human and animals and composed of 6 main species of B1, B2, G1, G2, M1 and M2. This categorization is correlated with the blue florescence formed under long wave UV light by B1 (Figure 1.4), B2 and green florescence formed under long wave UV light by G1, G2 in thin layer chromatography. Geographical conditions, agricultural applications, agronomic practices, and also susceptibility of the product for fungal growth during pre and post harvest period and conditions of storage are the main factors for the occurrence of Aflatoxin. Because of its high toxicity and carcinogenic effect; Aflatoxin, among all mycotoxin, has gained importance in recent years (Heathcote & Hibbert, 1978).

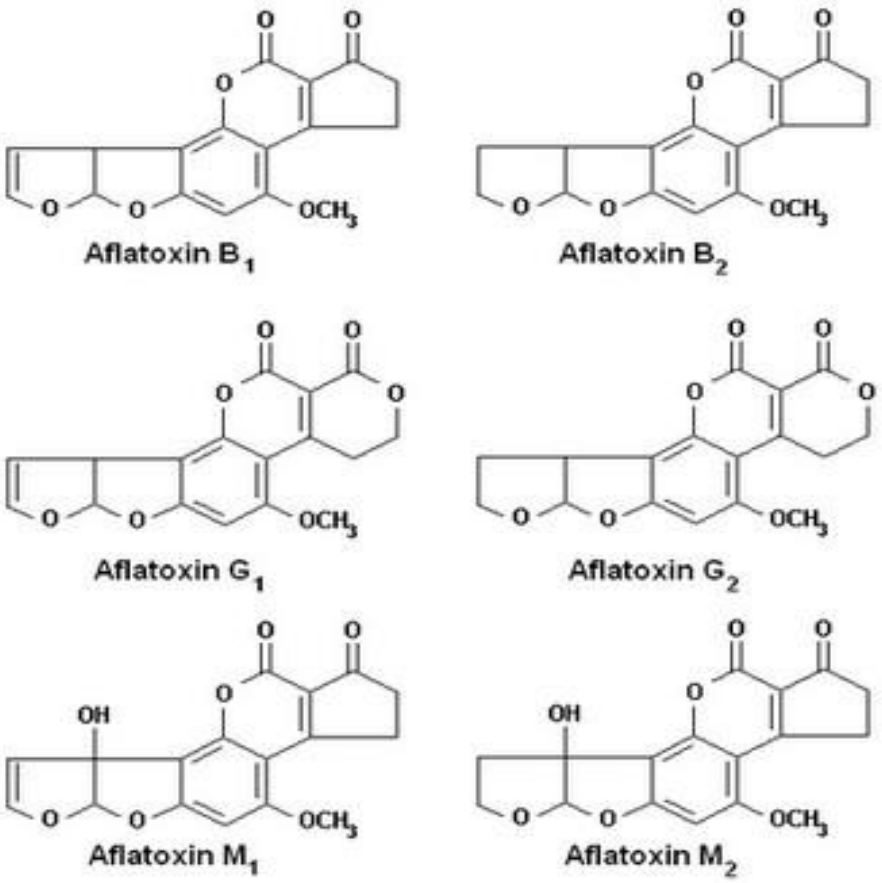


Figure 1.3 Chemical Structures of Aflatoxin B1, B2, G1, G2, M1, M2 (Wilson, 1990)

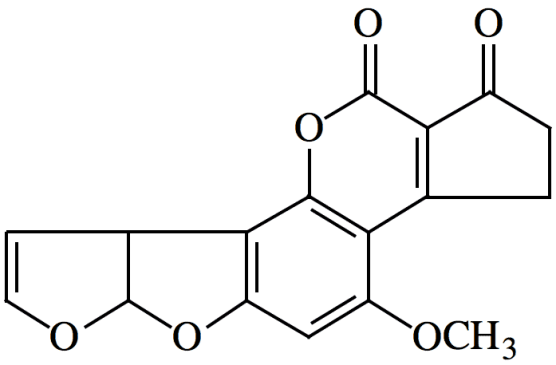


Figure 1. 4 Chemical Structure of Aflatoxin B1

Aflatoxin is not only dangerous for human health but also important in view of economic loss. The reports published by FAO also remark the excess of economic losses due to mycotoxin in the world. These product loss increase the economic losses of producer, loss of animal and milk, and negatively affect the distributors by rising cost, rising prices and health expenses.

Besides carcinogenic, mutagenic, and teratogenic effects, they are also important due to their resistance to heat treatments. Aflatoxins cannot be completely detoxified by various unit operations and thus possess risk to human health when present in animal feeds and even in small amount in meat, milk products and eggs (Karaman & Acar, 2006).Aflatoxin M1 and M2 are not directly produced by aflatoxigenic molds. These toxins are formed after the ingestion and metabolization of aflatoxin B1 and B2 contaminated feed products and found in milk of those animals. Aflatoxin producing *Aspergillus* species are extensively present all around the world and can grow easily under specific climatic conditions. Food products containing 9-14% or higher amount of water, can get moldy even in 3-4 days under the ambient temperature of 24-45˚C. *Aspergillus* species growth can cause danger in various food products such as maize, cotton seed meal, sunflower meal, soybean flour, nut, groundnut, walnut, oilseeds, fish and meat bone flour, that are raw materials used in the production of mixed feed.

According to the in vitro studies especially in human liver microsomes; the mechanism of AFB1 and AFM1 depend on the metabolic activation of epoxides which are the main principle of the difference of two toxins by means of potential of carcinogenicity, with the formation of primary metabolites. Like in the case of AFB1, epoxide conjugation of AFM1 is also with the reduced form GSH catalyzed by mouse, but not human liver cytosol. Although the binding mchanism of AFB1 to microsomal proteins depend on metabolic activation majorly; high level of AFM1 by microsomes are unrelated to metabolic activation. It appears possible that this property is related to the high cytotoxicity of AFM1. According to the in vitro studies using human cell; human cytochrome P450 enzymes in assays of acute toxicity have demonstrated a toxic potential of AFM1 in the absence of metabolic activation, in

contrast to AFB1. Especially during detoxification of AFM1, cytotoxicity as a biological response should be considered significantly (Neal, et.al, 1998).

1.2.1 Problems of Aflatoxin in Food Grains

Food safety is one of the major necessities in the world market. Quality and safety must be assured by food producers and governments also (Dowling, 1997). Mycotoxins in cereal products and the possibility of eliminating or reducing them to acceptable levels is one of the most important aspects of food safety. According to The Food and Agriculture Organization (FAO); mycotoxin contaminated cereal products are nearly 25% of the world total production (Dowling, 1997). Mycotoxins are one of the major causes of illnesses and high mortality rate of animals such as aflatoxins, deoxynivalenol (DON or vomitoxin) and fumonisins (Bullerman & Bianchini, 2007).

Aflatoxins are the naturally occurred highly toxic, mutagenic, teratogenic and carcinogenic substance and cause hepatic failure and hepatic carcinogenicity in human (Valeria, et.al, 1998). According to the International Agency for Research on Cancer (IARC, 2002) aflatoxins are classified as naturally carcinogenic to human. Variety of important agricultural products such as peanut, maize, rice, cottonseed, wheat are found to be contaminated by Aflatoxin mostly (Carvajal & Arroyo, 1997). Aflatoxin contamination leads to a great attention in recent years because of the high incidence rate in agricultural commodities, which leads to important economic losses.

There are several stages of harvesting that aflatoxin occur. Mostly; aflatoxins occur in crops in pre-harvest period. Postharvest contamination can be possible only if crop drying is delayed and moisture is allowed to exceed critical limits for the mold growth during storage. Insect or rodent infections facilitate mold invasion of some stored commodities (Liener, 1969).

Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices, and a variety of other foods and feeds (Figure 1.5). Because of the animal consumption of contaminated feed materials; milk, eggs, and meat products are also sometimes contaminated. However, mostly contaminated food commodities are corn, peanut and cottonseed, the reason of such high risk may be the conventional production and drying steps of corn, peanut and cottonseed in addition to that high nutritional value of these products may be another reason for mold growth.

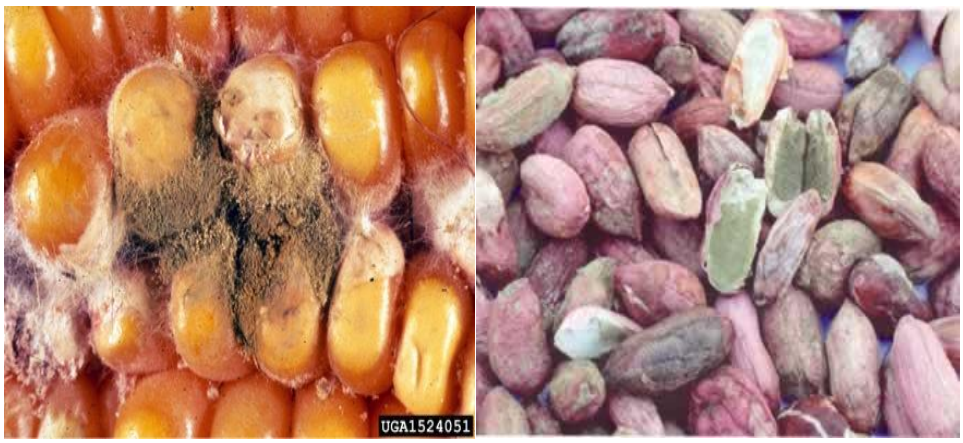


Figure 1.5 Mold Contaminated Corn and Peanut

Interactions of fungus and the host and the environment are important for fungal growth and aflatoxin contamination. Water and high-temperature stress, insect invasion to the host plant are the major factors in mold infestation and toxin production. As an example; high temperatures, prolong drought conditions, high insect invasion are the major aflatoxin contamination risk in pre-harvest period of peanut and corn. Aflatoxin production on peanut and corn in the postharvest period are favored by warm temperatures and high humidity (Liener, 1969)

1.3 Applied Methods for Detoxification

Detoxification of aflatoxin contaminated food and feed is an important problem, because of their high carcinogenicity and capability of staying unchanged through metabolic processes and massing in the tissues. Although numerous detoxification methods have been tested, only some of them seem to be able to fulfill the efficacy, safety, safeguarding measures of nutritional elements and costs requisites of a detoxification process. These methods can be divided into three subcategories, which are chemical, physical and biological techniques (Bozoğlu & Tokuşoğlu, 2011).

1.3.1 Chemical Detoxification

Detoxification, generally referred to chemical treatments, should be not only technically and economically feasible but also fulfill the criteria set by the FAO/WHO/UNEP conference on mycotoxins held in Nairobi, Kenya (1997). Destroying the toxin, reduction of the toxic properties of final product, destroying fungal spores, not producing toxic byproducts, not give damage to the structure and nutritive value of the product, and not changing technical properties of the final product are the basic principles and criteria of the detoxification processes.

Alkaline treatments are one of the major chemical treatments applied to the detoxification of Aflatoxin. Ammoniation and nixtamalization are the traditional alkaline treatments that applied to food and feed materials for detoxification and cause significant reduction of aflatoxin with the combination of heat treatment. Strong acids treatments convert aflatoxins to its hemiacetal form through hydration which are much less toxic. They found that the rate of conversion increased as the pH decreased or the temperature increased. According to the studies of Hamed (2006); aflatoxins were totally degraded under the steam processing of 129°C and 160 kPa with the addition of hydrochloric acid during manufacturing of vegetable proteins without production of toxic or mutagenic by-products (Hamed, 2006).

Bisulfite, is a highly reactive chemical agent used in food industry, with its high ability to denature enzyme and decrease non- enzymatic browning, decrease microbial growth and to act as an antioxidant and reducing agent. Bisulfite has also been tested for its ability to degrade aflatoxin. According to the study of Hamed (2006); sodium bisulfite was found to react with the double bond on terminal furan ring to make it more water soluble. B2 and G2 type aflatoxin does not have this double bond; hence, degradation by bisulfite is not possible. In order to form water soluble products; Sodium bisulfite react with aflatoxins (B1, G1, M1) under various temperature, concentration and time. Treatment of *Aspergillus flavus* inoculated groundnut cake with 1% sodium bisulfite at 10% moisture completely inhibited mold growth and aflatoxin production at room temperature (Hamed, 2006).

Methoxymethane formaldehyde, calcium hydroxide, ethylene oxide, hydrogen peroxide are the chemicals, commonly used in detoxification of food and feed materials. (Beckwith, et.al 1976). However; when the effect of chemicals on quality parameters (color, taste, etc.) are compared with the effect on aflatoxin, it does not seem feasible to apply chemicals with high doses to detoxify aflatoxin, but using them with other techniques may be more logical.

1.3.2 Biological Detoxification

Biological detoxification of mycotoxins works especially through two main mechanisms, sorption and enzymatic degradations, both of which can be achieved by biological systems. Live and dead microorganism can absorb and accumulate mycotoxins in their body or on their cell wall. Enzymatic degradation can be performed by either extracellular or intracellular enzymes and as a result of degradation. By this way; enzymatic modifications can change, reduce or completely eradicate toxicity (Aliabadi, et.al 2013).

A few strains of lactic acid bacteria (LAB) have been reported to bind Aflatoxin B1 and M1 in contaminated media or in a food matrix and prevent aflatoxins to transfer to the intestinal tract of humans and animals (Darsanaki, et.al., 2013).

Another biological method has been developed for the detoxification of aflatoxinB1 in a food matrix like peanut oil with use of abiological preparation from solid fermentation culture of *Aspergillus niger* F_{25} by using rice husk as carrier. At the dosage of 5-10%, BDA can reduce the level of AFB_1 in peanut oil from 20- 100 µg/kg to 1.9- 22.3 µg/kg at batch scale respectively. At this study, the optimal temperature and time for detoxification is 45-48°C and 2 hours respectively (Chen, et.al, 1998).

Although interesting and promising results are obtained from soil bacterium, LAB and some kind of enzymes, no biological system exists to be used in detoxification in full commercial sphere currently (Wu, et.al, 2009).The major drawbacks in using microorganisms are their utilization of nutrients from foods for their own growth and multiplication and release of undesirable compounds. One of the most practical approaches is the mixing of non-nutritive adsorbents with aflatoxin-contaminated feeds, which bind the toxins and inhibit their absorption from the gastrointestinal tract, thus minimizing the toxic effects to livestock and the carryover of these fungal metabolites into animal products (Ramos & Hernandez, 1997)

1.3.3 Physical Detoxification

Thermal treatments, gamma irradiation, extraction with solvents, adsorption are some of the physical methods for aflatoxin detoxification. The advantages of using physical techniques are that; they do not form other toxic compounds or leave any harmful residues, not seriously affect the nutritional quality of the products, mostly economically feasible and technically applicable (Rustom, 1997).

1.3.3.1 Thermal Detoxification

For heat processed food and feed materials; thermal inactivation is applicable for aflatoxin detoxification. Some of the mycotoxins are chemically stable at temperatures under 150°C (processing temperature), Aflatoxins are the most stable ones during heating and stay unaltered up to their melting point of around 250 °C (Yazdanpanah, et.al, 2005) and with thermal treatments applied to the food and feed materials such as boiling or autoclaving are not completely destroyed Aflatoxin.

Temperatures above 150°C are necessary for partial destruction of these toxins. The amount of destruction is dependent on the initial level of the contamination, type of toxin and food, heating time and temperature (Rustom, 1997)

In addition to these factors, moisture content, pH and ionic strength of the food are important parameters for toxin destruction. Moisture content is a critical factor, higher moisture content leads to easier inactivation of the toxin by heat.

In the case of peanut; the minimum moisture content is about 8-10% and moisture content of about 15-35% are the optimum values for aflatoxin production on peanut (Jankhaikhot, 2005) Broken, undersized, rancid and discolored peanut kernels are most likely to be contaminated. The physical damage leads to an increase of hygroscopic characteristic of peanuts during storage, thus leads to a higher incidence of mold growth.

It is debatable that the moisture content has an effect on helping the opening of lactone ring in AFB1 to form a carboxylic acid. Lactone ring with its terminal acid group undergoes heat- induced decarboxylation. In addition to the moisture content, the presences of ionic salts increase degradation of aflatoxin by heat (Rustom, 1997).

There are several studies about the aflatoxin detoxification in peanut and peanut meals. These studies showed that; aflatoxins may partially be destroyed by oil and dry roasting of peanuts. According to the studies of Stoloff (1984) and Baur (1975);

aflatoxin in peanut samples are stable at room temperature, and also there is no change in the levels of aflatoxin at room temperatures up to 2 years. As an alternative technique; Lutter (1993) reported that, microwave roasting destroys aflatoxins completely.

According to some studies on peanuts, when contaminated peanuts with an initial aflatoxin levels of 2200-4100 µg/kg were dry roasted at 121°C for 30 minutes, destruction levels were 84- 85% respectively. However when the contaminated peanuts with initial level of 370-317 µg/kg were dry roasted at 150°C for 30 minutes, destruction levels were limited to 48-47% (Rustom, 1997).
Another study showed that; roasting of peanut samples at 120°C for 120 minute and 150°C for 30-120 minutes causes substantial reduction on the level of aflatoxins in samples. About 90% of the aflatoxins were destroyed in the process of 120 minutes at 150°C with initial aflatoxin levels of 44 ppb AFB1 and 5.6 ppb AFB2 (Yazdanpanah, et.al, 2005).

According to the study on Nigerian peanuts; researchers aimed to correlate loss of aflatoxin positively in the products at the roasting conditions. Seeds are dry roasted at 140°C for 40 minutes resulted in 58.8% and 64.5% reductions in AFB1 and AFG1, at 150°C for 25 minutes resulted in 68.5% and 73.3% reductions in AFB1 and AFG1 respectively. The maximum reduction levels were observed at 150°C for 30 minutes as 70% and 79.8% in AFB1 and AFG1 respectively. In this study; initial level of aflatoxin B1 was ranged between 1.44 to 2.24 ppm and aflatoxin G1 was ranged between 1.10 to 2.58 ppm (Ogunsanwo, et.al, 2004).

1.3.3.2 Gamma Irradiation

Mycotoxins are highly toxic, so it is important to reduce their levels in food and feed as low as technologically feasible. Ionizing radiation is one of the major techniques applied for this purpose. It has not only effect on mold and fungus viability but also

production of mycotoxins on food and feed materials. In addition to that; under specific conditions; ionizing radiation can have a direct action on elimination of mycotoxins (Calado, et.al, 2014).

There are several studies about the effect of gamma irradiation on detoxification of mycotoxins. Some of the studies claimed that radiation in general do not have an effect on aflatoxin detoxification. According to the studies of Shantha & Murthy (1977), ultraviolet light and gamma irradiation were not efficient on reducing aflatoxin levels in feed and foodstuffs (Shantha & Murthy, 1977). On the contrary to their studies, sunlight is observed as effective on detoxification of aflatoxin in oil type food materials (Samarajeewa, et.al, 1990). Unrefined groundnut oil containated with aflatoxin was subjected to direct sunlight for one hour in glass containers (approx.50000 lux), the aflatoxin was almost completely eliminated. It was confirmed that sunlight exposed oil were safe and stable in terms of aflatoxin (Temcharoen & Thilly, 1982).

In crude groundnut oil, aflatoxin in finely suspended form can easily be separated by filtration. Special filter pads are effective on removal of aflatoxin from crude oil (Shantha & Murthy, 1977).

In the study of Rustom (1996); 75 to 100% reduction of AFB1 was achieved after the application of 1 to10 kGy gamma irradiation on peanut meal respectively. The presence of water has an important role in the destruction of aflatoxin by gamma energy, because of the radiolysis of water causes to the formation of highly reactive free radicals. These radicals can react with the terminal furan ring of Aflatoxin B1 and giving products of lower biological activities.

In order to increase the efficacy of the gamma irradiation, hydrogen peroxide was added to the sample in aqueous form. Addition of 1 ml of 5% hydrogen peroxide to an aqueous AFB1 solution (50µg/ml) resulted in 37% degradation of the toxin at 2 kGy as a lower dose of gamma irradiation (Temcharoen & Thilly, 1982).

In order to analyze the synergistic effect of hydrogen peroxide and gamma irradiation, the effect of pH on the inactivation of aflatoxin was checked by taking 5% (wt/vol) hydrogen peroxide (H_2O_2) in aqueous system at different pH values. Hydrogen peroxide at 1% and 3% yielded 37% and 43% inactivation of aflatoxin respectively at 2 kGy. Increasing the dose to 10 kGy did not significantly affect the reduction level of aflatoxin, however; 5% hydrogen peroxide caused 100% inactivation of aflatoxin at a level of 2 kGy gamma irradiation (Patel, et.al, 1989).

In the study of Ghanem, Orfi, & Shamma (2008); food and feed samples were irradiated at doses of 4.6 and 10 kGy. Results showed that degradation of AFB1 was positively affected with the increase in the applied dose of gamma rays. The initial amount of AFB1 level in the samples was 16 mg/kg (16000 ppb). At a dose of 10 kGy gamma irradiation; degradation level of aflatoxin was reached to 58.6%, 68.8%, 84.6%, 81.1% and 87.8% for peanuts, peeled pistachios, unpeeled pistachios, corn and rice respectively. In peanuts; which contained highest oil content, percentage of AFB1 degradation at 10 kGy was about 58.6% however, the corresponding value in rice which had the lowest oil content reached the highest value as 87.7%. The result of this study indicated that; there is a controversial correlation between oil content of the sample and aflatoxin detoxification during gamma irradiation.

1.4 Effect of Applications on Protein Content of Samples

The food industry is increasingly focused on the analysis, certification/ documentation and characterization of the raw materials and ingredients that are used in foods, in addition to analyzing the quality of the final food product. The product obtained after different processes should be not only microbial safe and aflatoxin free but also high quality. In order to achieve these; chemical composition, mineral content, protein and lipid content of the samples are analyzed before and after processing.

According to the study of Damame, et.al, (1990); in 160°C/ 30 minutes roasted and 150 days stored at 27°C and 5°C untreated peanut kernels, methionine, tryptophan and in vitro protein digestibility were significantly decreased. In addition to that; there is a significant increase in the levels of soluble proteins and acid value of kernel oil. The storage of heated peanuts at 5°C was found to be effective on lowering the undesirable nutritional changes in the peanut kernels.

Same analyses were made with microwave oven roasting for peanut meal. The results revealed that roasting of peanut by microwave is better than ordinary roasting in maintenance of chemical composition and minerals contents. In addition, it raised protein efficiency ratio (PER is the ratio of grams of body weight gain (in specified time) to the grams of protein consumed) of peanut protein more than that of ordinary roasting (El- Badrawy, et.al, 2007).

In the study of Chen & Phillips (2005); partially defatted peanut flour was processed in a twin-screw extruder. Results showed that; solubility of peanut flour protein increased up to 10%.

There are several techniques to analyze the change in the structure of protein, and also its amount. Some of these techniques are for the determination of overall protein content such as Kjeldahl Method. In addition to them; there are some instrumental techniques such as UV- visible spectroscopy, ion exchange and affinity chromatography. One of the most common used techniques for instrumental techniques is Fourier transform infrared spectroscopy. Unlike X-ray crystallography and NMR spectroscopy which provide information about the tertiary structure, FTIR spectroscopy provides only information about the primary and secondary structure content of proteins (Gallagher, FTIR Analysis of Protein Structure).

1.4.1 Fourier Transform Infra-red Spectroscopy Analyses for Protein Profiles

Fourier transform infrared spectroscopy (FTIR) is a technique which collects data in a spectral range and used for obtaining an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas.

In order to analyze chemicals whether organic or inorganic structure, FTIR is an useful technique and it can be utilized to quantitate some components of an unknown mixture. Working principle depend on the molecular vibration of the samples. Molecular bonds vibrate at different frequencies depend on type of bonds and elements that it contains. According to the quantum mechanics; lower frequencies correspond to the ground state and higher frequencies correspond to the excited sates of bonds. Exciting by light energy is one of the major ways to cause molecular vibrations by having it absorb light energy. For any given transition between two states, the light energy (determined by the wavelength) must exactly equal the difference in the energy between the two states (Fourier Transform Infrared Spectroscopy, 2014).

Figure 1.6 reveals the Amide I and Amide II regions which are characteristic bands found in the infrared spectra of proteins and polypeptides (Figure 1.6). These bands arise from the peptide bonds that link the amino acids.

Designation	Approximate frequency (cm^{-1})	Description
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1600–1690	C=O stretching
Amide II	1480–1575	CN stretching, NH bending
Amide III	1229–1301	CN stretching, NH bending
Amide IV	625–767	OCN bending
Amide V	640–800	Out-of-plane NH bending
Amide VI	537–606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

Figure 1. 6 Characteristic Infra-red Bands of Peptide Linkages (Kong & Yu, 2007)

The absorption associated with the Amide I band may correlate with stretching of the C=O bond of the peptide linkage and Amide II band may correlate with the bending of the N—H bond. Both C=O and N-H bonds consist of hydrogen bonding in the secondary structure of proteins, the places of Amide I and II are depend on the protein content. Studies with proteins of known structure have been used to correlate systematically the shape of the Amide I band to secondary structure content. Although, the Amide II band is sensitive to secondary structure content, it is not a good predictor for quantitating the secondary structure of proteins (Figure 1.7).

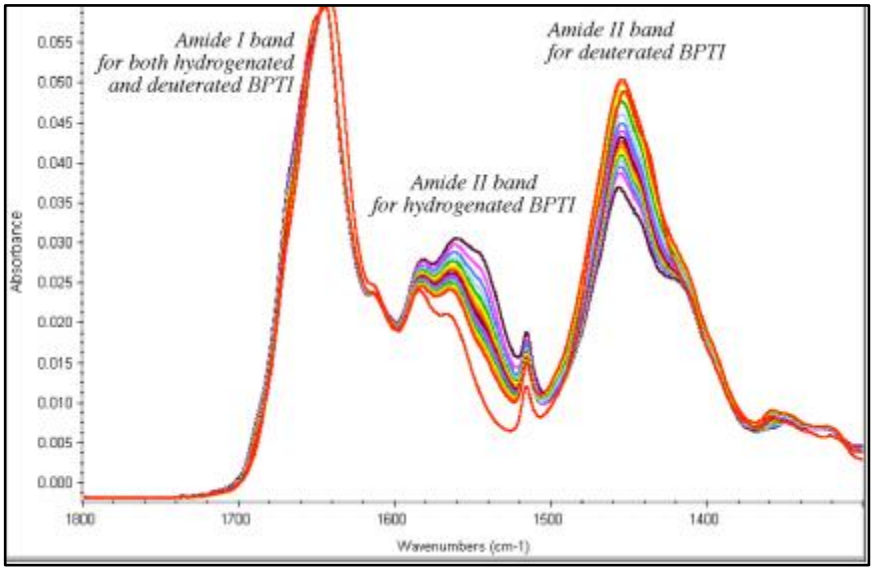


Figure 1. 7 The Sample Spectra of Amide 1 and Amide II Bands
(Gallagher,2014)

1.5 Analyses of Aflatoxin B1 Toxicity

Aflatoxins are hepatotoxic and hepatocarcinogenic agents. They are also mutagenic, teratogenic, and cause immunosuppression in animals. Liver is the principal organ affected by aflatoxin, but high levels of aflatoxin have also been found in the lungs, kidneys, brains and hearts of individuals dying of acute aflatoxicosis. Lethal dose values for animals vary between 0.5 and 10 mg/kg body weight (Lawley, 2013). Toxic response depends on how the molecule is metabolized in the liver. For adult male rat 7.20 mg/kg, adult dog 0.50 mg/kg and day-old duckling 0.35 mg/kg body-weight Aflatoxin B1 cause hepatitis B infection. No animal species is resistant to the acute toxic effects of Aflatoxin B1 (Saini & Kaur, 2012).

In response to acute or chronic toxoplasmosis; most of the liver enzymes especially glutathione peroxidase as hepatic antioxidant show a significant increase and playing a significant role in detoxifying lipid peroxide and hydrogen peroxide (Al-Taee & Hassan, 2012).

The hepatotoxicity of the aflatoxin is thought to be mediated by their ability to generate reactive oxygen species and cause oxidative damage. The study of Kheir According to the study of Eldin, et.al, (2008); ability of some natural antioxidants such as; vitamin E and Se, β- carotene, silymarin and coenzyme Q10 on Aflatoxin B1 induced hepatotoxicity in rat models. There is an increase of alanine, aspartate aminotransferases and alkaline phosphatase (ALP) observed in the serum of rats administered with 250 ppb AFB1/ day for 2 weeks. In addition to these results, no significant change was detected in the activities of glutathione peroxidase (GPx), glutathione reductase (GR) and cytochrome c-reductase. On the other hand, significant increase of glutathione -S- transferase (GST) levels and decrease in glutathione (GSH) were observed (Kheir Eldin, et.al, 2008).

In addition to these results; according to the study of Devendran & Balasubramanian (2011) rats administered with different levels of aflatoxin (20, 40, 60, 80,100 ppm) for 8 days; significant reduction in the activities of catalase, superoxide dismutase and glutathione peroxidase (GPx) as well as glutathione reductase (GR) in the liver and kidney of rats were observed (Figure 1.8).

Groups	LDH	SOD	CAT	GPX	GST	GR	LPO	ALP	G-6-ph	Fru-1-6 bisphos
Normal	20.0 ± 1.46	4.80 ± 0.09	36.90 ± 0.46	4.00 ± 0.014	0.90 ± 0.008	0.80 ± 0.009	1.60 ± 0.008	160	12.10	11.90
20 ppm	18.0 ± 1.80	3.60 ± 0.08	30.92 ± 0.49	4.00 ± 0.018	0.82 ± 0.007	0.72 ± 0.004	1.49 ± 0.08	190	10.00	11.00
40 ppm	18.5 ± 0.89	3.40 ± 0.06	26.90 ± 0.56	2.91 ± 0.016	0.65 ± 0.006	0.60 ± 0.006	2.33 ± 0.005	200	9.60	11.00
60 ppm	12.0 ± 0.42	2.40 ± 0.07	20.85 ± 0.38	2.90 ± 0.026	0.60 ± 0.004	0.40 ± 0.008	2.25 ± 0.004	260	8.00	10.50
80 ppm	10.9 ± 1.02	2.20 ± 0.01	16.80 ± 0.28	1.60 ± 0.019	0.50 ± 0.006	0.20 ± 0.007	4.19 ± 0.06	300	6.00	9.00
100 ppm	10.5 ± 1.03	1.10 ± 0.05	10.70 ± 0.26	0.50 ± 0.011	0.45 ± 0.005	0.10 ± 0.005	3.08 ± 0.003	290	6.20	9.00

Figure 1. 8 Effect of Aflatoxin Induced Changes in The Enzyme Parameters of Rat Liver (Devendran & Balasubramanian , 2011)

On the contrary to the results obtained from the study of Kheir Eldin, et.al. (2008)another study indicates that; enzyme activities of glutathione peroxidase and

glutathione reductase were significantly decreased in rats fed with 50 ppm aflatoxin. According to the same study; melatonin exhibited an efficient protective effect against aflatoxin B1 and clinical application of melatonin may be considered in cases of aflatoxicosis (Ar, et.al., 2001).

1.6 Analyses on Changes of Aflatoxin Structure

AFB1 is not mutagenically active by itself. However, because of some systematical oxidation- reduction mechanisms, it becomes toxic. It is primarily metabolized at the liver to metabolites like steroids or xenobiotics, and has several metabolites such as aflatoxicol, aflatoxicol H1, and AFQ1. There are three stage of the metabolism of aflatoxin like lipophilic compounds. It contains the activation of AFB1 by cytochrome P450 dependent monooxygenase (CYP) that is involved in phase I metabolism (Dutton, 1988).

The amount and structural changes of the aflatoxin can be analyzed by several methods as mentioned above. One of the novel techniques about aflatoxin detection and structural changes analyses was FTIR.

It is essential to obtain a calibration between IR band intensity and aflatoxin content in order to analyze the Aflatoxin level by FTIR. By using the calibration curve, the amount of the unknown sample can be estimated. Comparison of recorded spectra of the sample under the same conditions with the calibration and the standards used are used for analyses with FTIR.

In order to analyze the changes in aflatoxin structure Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) was used because it allows analyzing liquid samples and thin films. The main distinction of ATR-FTIR; it depends on total internal reflection and attenuation of a total reflection and main advantage of this system is a minimum or no sample preparation (Mirghani, et.al, 2001).

Using spectral rationing to analyze small differences is one of the most important strengths of FTIR. Aflatoxin exhibited characteristic absorption bands at wavelengths; 1440-1500 cm^{-1} for H-C-H bending, 1200- 1300 cm^{-1} for C-O, 1720-1745 cm^{-1} for C=O ad 1450- 1500 cm^{-1} for C=C.

FTIR spectral data of varying levels aflatoxin contaminated peanut paste samples were preprocessed and normalized with logarithmic method and used for the partial least square regression for training sample sets created by spiking known amount of aflatoxins with clean peanut paste (Kaya- Çeliker, et.al, 2011). According to these studies; aflatoxin amount were analyzed in the spectral regions 1584-1484 and 1424-1127 cm^{-1}.

According to another study performed by Mirghani, et.al, (2001); 3004-2969 cm^{-1} wavelength interval corresponds to the absorption bands for CH_2, aromatic =CH, -C-H, C=C and phenyls, 1744- 1720 cm^{-1} wavenumber interval corresponds to C=O, 1364-1369 cm^{-1} for methyl adjacent to epoxy ring, 1217-1220 cm^{-1} interval for in plane -CH bending of phenyl, 1035-1037 cm^{-1} for symmetric streching of =C-O-C or symmetric bending of phenyl, and 900-902 $cm^{-1}$for possibly isolated H.

1.7 Aim of the Study

Aim of the study was to reduce the Aflatoxin B1 content in peanut meal by roasting at 120°C for 30, 60, 90 minutes, 160°C for 30, 60 minutes and 200°C for 5 minutes and gamma irradiation at 10 kGy.

The changes in the Aflatoxin B1 structure were analyzed, to understand whether the new formed structures were more toxic than Aflatoxin B1. In order to determine the toxicity; liver enzyme glutathione peroxidase activity was studied. Inhibition mechanism of the glutathione peroxidase gave idea about structural differences and inhibition mechanism of Aflatoxin B1 (Kheir Eldin, et.al, 2008).

According to the literature; protein content of the peanut meal are important as feed material. Because of that; the changes on protein content of peanut after roasting and gamma irradiation become important. FTIR analyses were performed for protein studies (Mirghani, et.al, 2001)

This study reveals the results on the effect of roasting as thermal treatment and gamma irradiation on the detoxification of Aflatoxin B1, change in the structure of Aflatoxin B1 and its toxicity and change in the protein content of peanut meal samples and compare with the literature.

CHAPTER 2

MATERIALS AND METHOD

2.1 Experimental Materials and Locations

The roasting experiments were conducted in Research Laboratory in Food Engineering Department of METU and aflatoxin analyses were done in İzmit Food and Control Laboratory under management of Ministry of Food Agriculture and Livestock.

Peanut meal feed materials were obtained from Osmaniye originated feed factory. Moisture content of peanut was 8.62±0.45% in average. The average grain size after milling varied between 100-150 μm for fully defatted peanut flour (Figure 2.1).

Figure 2. 1 Peanut Meal

2.2 Sample Preparation

2.2.1 Sample Preparation for Dry Oven Roasting

Peanut meal was milled in laboratory size mill (Thomas& Wiley, Model 4). After milling, moisture content of the samples was analyzed by moisture analyzer (A&D, MS20). The value of the moisture content in average was 8.62±0.45%. During experiments moisture of the samples were adjusted to 20.00± 0.51%. So, samples were humidified with the addition of distilled water.

During analyses, samples were divided as chemically treated and non-chemical treated ones. NaOH and $Ca(OH)_2$ (Sigma Aldrich) were used as chemicals. The amounts of chemicals were set as 3& 5% for NaOH and 0.2& 0.8% for $Ca(OH)_2$.

2.2.2 Sample Preparation for Gamma Irradiation

The peanut meals were put into small bags and placed in opaque boxes. The boxes were sent to the Turkish Atomic Energy Authority (TAEA) and irradiated in Sarayköy Nuclear Research and Training Centre (SANAEM).

2.3 Gamma Irradiation Treatment

In TAEA; the boxes (45*45*90 cm size) are transported to the irradiation room by horizontal conveyors.

The irradiation treatment took place in this room and samples passed across the gamma source (Cobalt-60) and absorbed the radiation. The process continued till reaching the desired absorbed dose (10 kGy) for samples (nearly 24 hours).

2.4 Roasting Processing

After milling the samples, moisture of them was analyzed, chemical addition was applied and samples were humidified till 20%.

Roasting was applied in a lab scale oven. Heating of samples were carried in glass cups submerged in heat controlled oil bath. Heating is done at 120°C for 30-60-90 minutes, at 160°C for 30-60 minutes and at 200°C for 5 minutes. Samples were mixed continuously in order to obtain a homogenous heating.

After roasting, cooled samples were sealed in plastic bags and stored at refrigeration temperature till aflatoxin analyses.

2.5 Capillary Tube Heating

Constant amount of aflatoxin (15 µl) were heated by capillary tubes in a heat controlled oil bath at 120°C for 30-60-90 minutes, at 160°C for 30-60 minutes and at 200°C for 5 minutes. The diameters of the capillary tubes were 0.49 mm.

2.6 Aflatoxin Determination Analyses

As mentioned in Part 2.1, aflatoxin analyses were performed in İzmit Food and Control Laboratories. The technique used in this laboratory is accredited for aflatoxin analyses in peanut meal *(AOAC Official Method 991.31).* The steps of the technique

was given below and detailed information used during analyses were given in Appendix E.

- Weighting of 25 gr sample
- Milling
- Addition of 125 ml methanol/ water solution (70/30)
- Shaking 2 minutes
- Filter with Whatman 4 paper
- Taking 15 ml of filtered sample and add 30 ml distilled water
- Taking 15 ml of prepared solution and add 10 ml distilled water
- Separation of aflatoxin by immunoaffinity colons (AFLAPREP immunoaffinity columns were used for detection) (Appendix E/ Figure E2)
    - *The columns contain a gel suspension of monoclonal antibody specific to the toxins of interest. Following extraction of the toxins the sample extract is filtered, diluted and passed slowly through the immunoaffinity column. Any toxins which are present in the sample are retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxins are then released from the column following elution with solvent. The eluate is collected prior to analysis by HPLC or LC-MS/MS. Aflatoxins are required to be derivatised when analysed by HPLC (r-biopharm).*

  Recovery by;
- Addition of 1 ml methanol
- Addition of 1 ml of distilled water

After samples were taken into the vials, HPLC (Agilent 1100) was used for aflatoxin analyses. HPLC parameters were given in Appendix E.

2.7 Protein Content and Aflatoxin Structure Analyses

The aim of these analyses is to determine the effect of different application (roasting and gamma irradiation) on the total protein content of the peanut meal. Ground samples were roasted at 120°C for 30,60 and 90 minutes, at 160°C for 30 and 60 minutes, 200°C for 5 minutes and gamma irradiated. After the applications; by obtaining the graphical representation of FTIR absorption curve vs. wavelength number, protein content of the samples were analyzed by IR Affinity-1 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan)in 400-4000 nm wavelength interval at a resolution of 4 cm^{-1} with 32 scan for FTIR experiments (Figure 2.2)

Figure 2. 2 Fourier Transform Infra-red Spectroscopy

2.8 Toxicity Analyses of Aflatoxin

Liver antioxidant enzyme, glutathione peroxidase activity assay was used to see ifany increase or decrease of the aflatoxin toxicity occurs after applied processes. Glutathione peroxidase's main activity is to protect the organism from oxidative

damage. The biochemical functions of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water and its activity is effected by the presence of aflatoxin.

Enzymatic analyses were performed at 37°C. Reaction took place in 500µL of 50 mM potassium phosphate buffer, pH 7, containing 1 mM EDTA, 1 mM sodium azide and 1 mM GSH (Sigma Aldrich), 1 unit of GSSG reductase and 10 µM of enzyme. The enzyme was preincubated for 7 min. and then 0,25mM NADPH solution was added and incubated for 3 minutes at 37°C.The activity was monitored at 340 nm at 20 second by spectrophotometric methods (Shimadzu UV-1700) (Yu, et.al, 2005).

2.9 Statistical Analyses

The analysis of regression was carried out to investigate the effect of operating conditions on the final treated product quality and aflatoxin content using MINITAB (Version 16). Multi way ANOVA (analysis of variance) were used for comparison of means. Significance was accepted at 0.05 level of probability ($p<0.05$). Mean separation was performed by Tukey Test for multiple comparisons of means. All physical and chemical measurements were performed in triplicate.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Chemical Composition of Peanut Meal

During heating and gamma irradiation, chemical composition of the samples became important parameters for inactivation of aflatoxin(Samarajeewa U.,et.al, 1990). In peanut meal; because of extraction of peanut oil, free radical formation due to gamma irradiation and roasting is lowered. The reasons of free radical formation in gamma irradiation and thermal treatment are radiolysis and thermolysis, respectively (Horváthová, et.al, 2007). In addition to that; peanut meal contain high amount of arginine, histidine as essential amino acids and also tryptophan (Kholief, 1987). These amino acids have basic and non-polar structure (Dept. of Biol., Penn State, 2002).So, the nature of these amino acids became important for the selection of type of chemical treatment (acid or base). In this study; because of the basic nature of the protein structure of peanut meal; basic chemical treatment instead of acidic treatment was preferred for not to degrade the quality parameters (such as color) and digestive properties also (Grosso, et.al,1999).

Before the experiments, samples were analyzed for moisture, protein, and carbohydrate and fat contents. According to the results of the analyses; the moisture content, protein content, carbohydrate content, fat content of the samples were 8.62±0.45%, 50.21±0.02%, 40.05±0.25%, 2.00±0.01% respectively and ash content was 2.55± 0.01%.

3.2 Effect of Roasting on Aflatoxin B1 Level in Peanut Meal

Detoxification of Aflatoxin B1 (herein after represented as B1 in the graphs) in peanut meal by roasting is presented in Figure 3.1- 3.4.

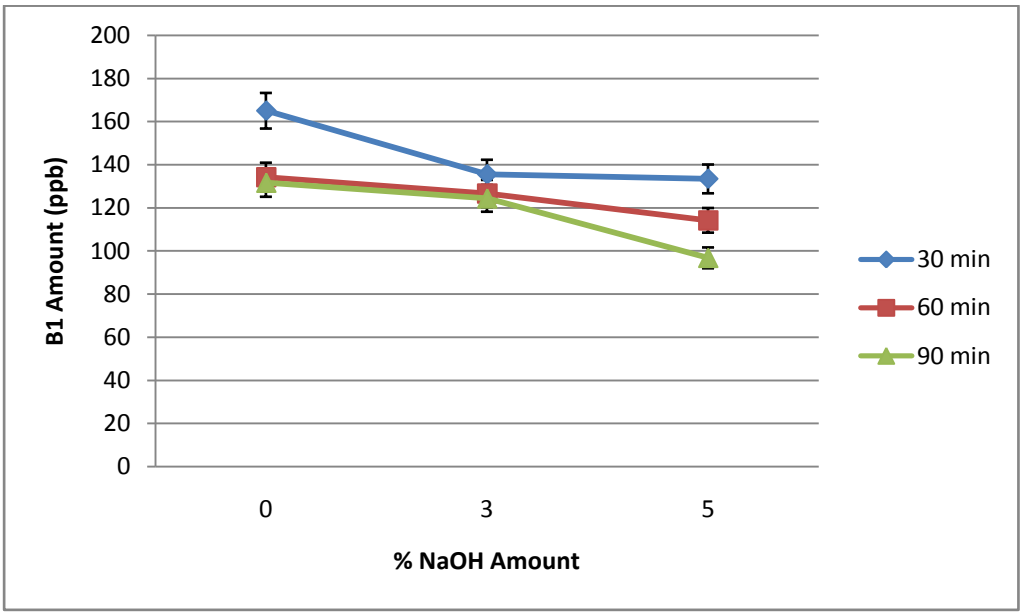


Figure 3. 1Effect of Dry Heating at 120°C on Aflatoxin B1 in Peanut Meal with NaOH Addition

In Figure 3.1; detoxification of Aflatoxin B1 contamination on naturally contaminated ground peanut samples at 120°C with the addition of 3% and 5% NaOH is given. Natural Aflatoxin B1 content of peanut meal samples was 185.00±1.04 ppb. Although all treatments showed some degree of Aflatoxin B1 detoxification, samples roasted at 120°C for 90 minutes with the addition of 5% NaOH had the maximum aflatoxin B1 reduction as 52%.

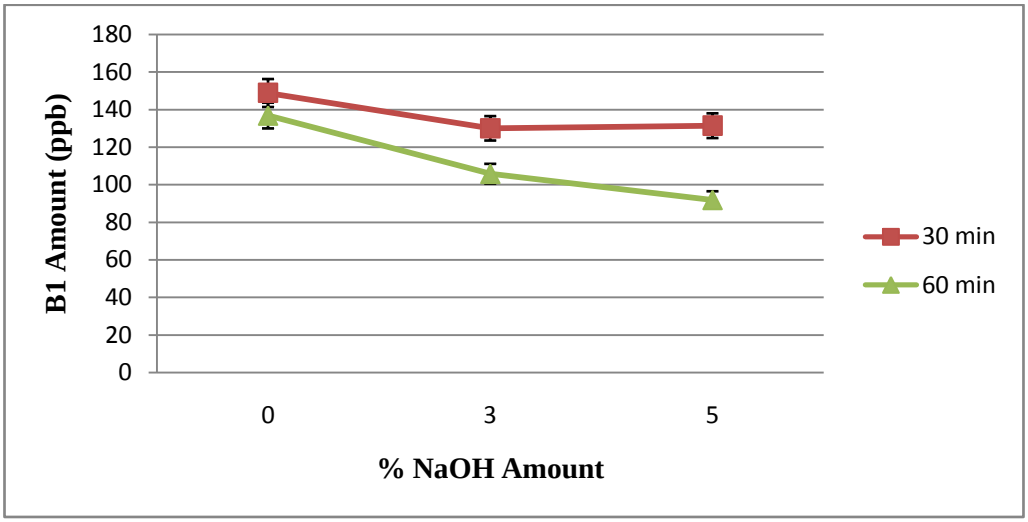


Figure 3. 2 Effect of Dry Heating at 160°C on Aflatoxin B1 in Peanut Meal with NaOH Addition

In Figure 3.2; detoxification of Aflatoxin B1 contamination on naturally contaminated ground peanut meal at 160°C with the addition of 3% and 5% NaOH is presented. As can be seen from the Figure 3.2; maximum reduction as 50% was observed at 160°C roasting with 5% NaOH addition for 60 minutes. Because of the changes in physical appearance of peanut meal samples heating at 160°C for 90 minutes was not carried.

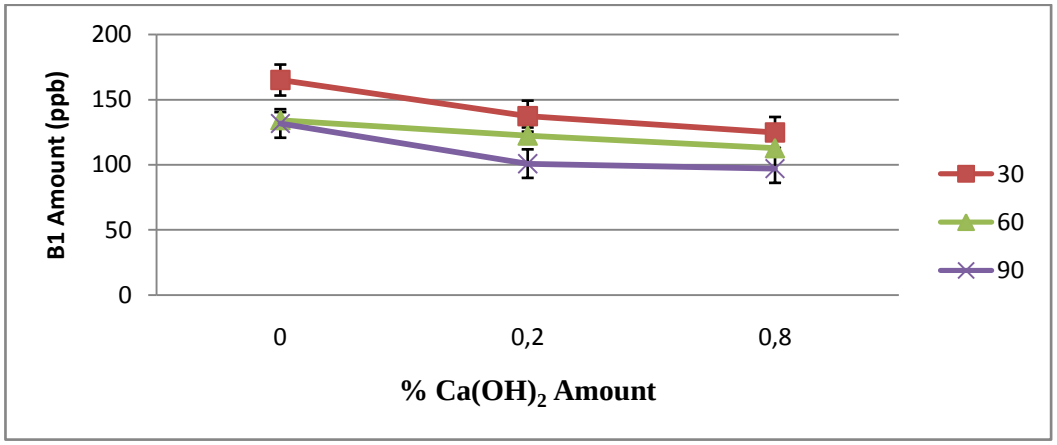


Figure 3. 3Effect of Dry Heating at 120°C on Aflatoxin B1 in Peanut Meal with $Ca(OH)_2$ Addition

In Figure 3.3; detoxification of Aflatoxin B1 contamination on naturally contaminated ground peanut meal at 120°C with the addition of 0.2% and 0.8% $Ca(OH)_2$ is presented. Maximum reduction was obtained as 52% at 120°C for 90 minutes with the addition of 0.8% $Ca(OH)_2$.

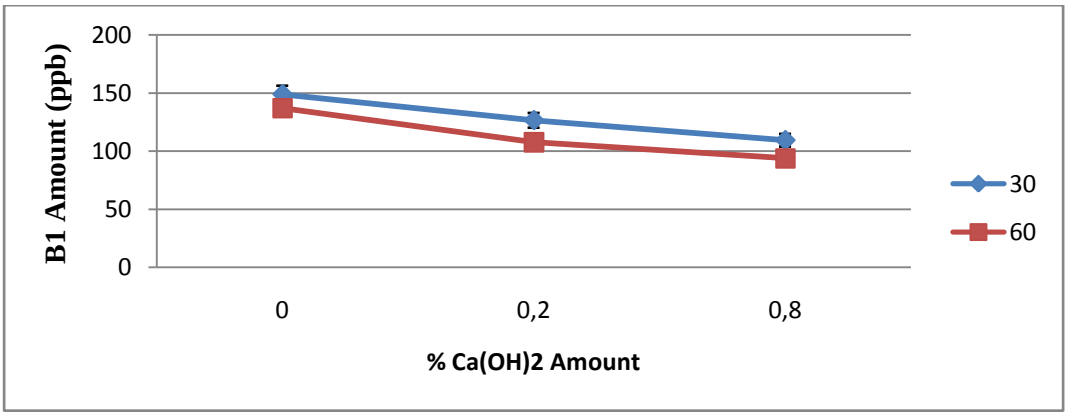


Figure 3. 4Effect of Dry Heating at 160°C on Aflatoxin B1 in Peanut Meal with $Ca(OH)_2$ Addition

In Figure 3.4; detoxification of Aflatoxin B1 contamination on naturally contaminated ground peanut meal at 160°C with the addition of 0.2% and 0.8% $Ca(OH)_2$ is presented. Maximum reduction was obtained at 160°C for 60 minutes with 0.8% $Ca(OH)_2$ addition as 51%.

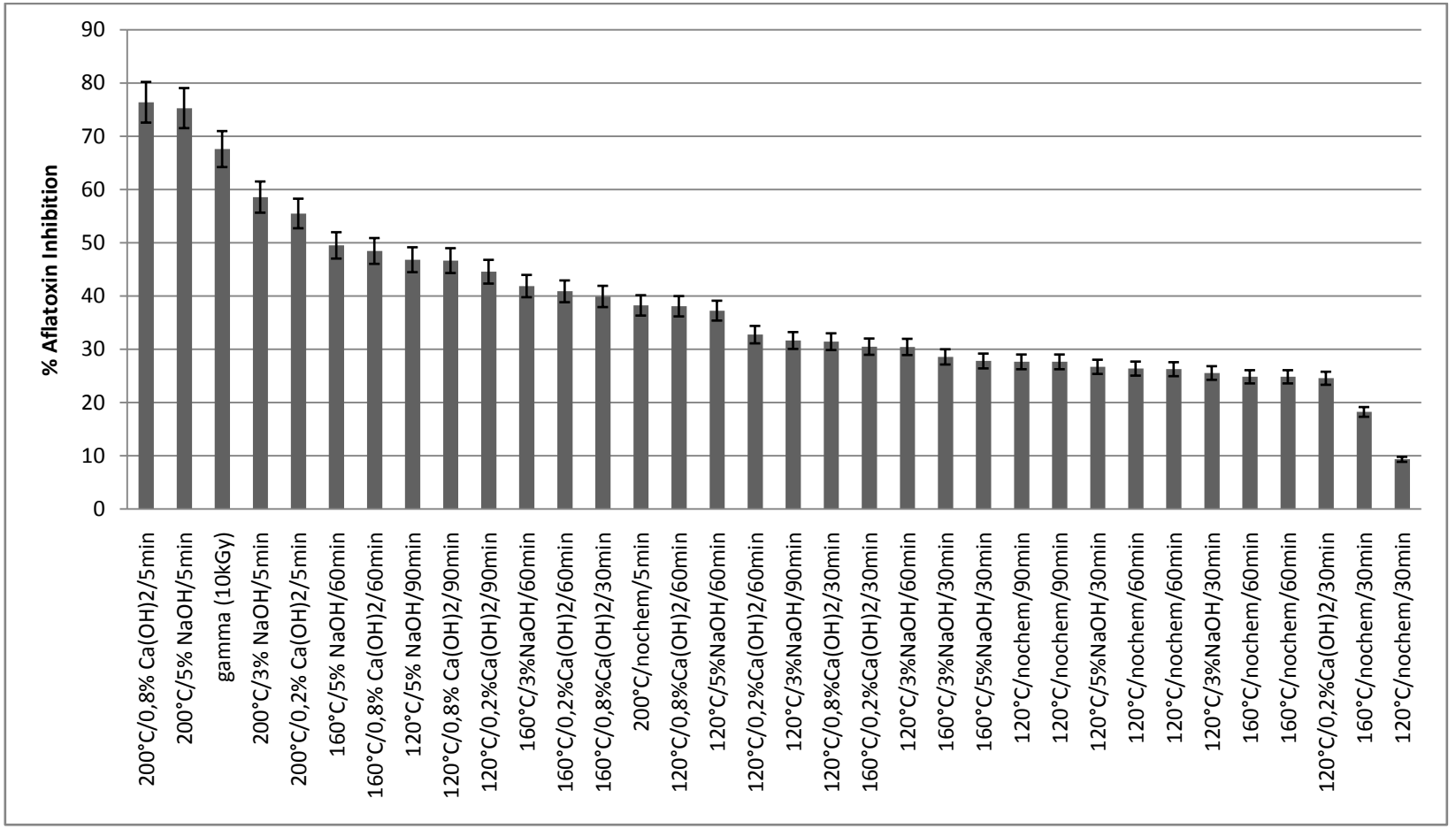
All data obtained from roasting applications are given in Appendix F and all the % reduction values are given in Table 3.1.

According to the results given in tables in the appendix F; maximum reduction was obtained during roasting at 200°C for 5 minutes with addition of 0.8% $Ca(OH)_2$ as 77%. Roasting samples for 90 minutes is practically difficult, so roasting at 200°C for 5 minutes can be more applicable in industrial scale not only for avoiding wasting time but also being economically efficient. Since, physical changes (color change and burning) of peanut were observed at roasting temperature of 200°C after 5 minutes, processes at 200°C for longer times were not considered.

According to the study of Rustom (1997); the presence of ionic salts resulted an increase of aflatoxin degradation by heat. They observed that; peanut meal treated with 5% NaCI solution heated at 116°C for 30 minutes resulted reduced content of aflatoxin by 80-100% more compared to unsalted control samples. The figures above and the data given in Appendix F, percent reduction of Aflatoxin B1 with no chemical addition samples and chemical added samples are compared. According to the results; at 120°C roasting; in both NaOH added and $Ca(OH)_2$ added samples; 20±2% more detoxification was observed. Same case is valid for 160°C roasting, 25±3% more detoxification was observed for both chemical addition .According to the ANOVA statistical analyses and Tukey test (Appendix D); chemical type and chemical amount were not significantly effective variables on detoxification of Aflatoxin B1 ($p \geq 0.05$), however temperature and time were significantly effective variables ($p \leq 0.05$). At this stage; it can be discussed that; the reason of more reduction was not due to the type or amount of chemical addition but because of the presence of ionic salts, which can be explained by the same mechanism discussed as the thermolysis of water molecules and reaction of free radicals with the double

bonds in the cyclic structure of the Aflatoxin B1 molecules (Rustom, 1997). Although there is no given mechanism in the literature on the ionic salts inactivation; the predicted reason (Rustom, 1997) may be the proposed mechanism of detoxification.

Table 3.1 % Aflatoxin Inhibition vs. Applied Techniques



3.3 Effect of Capillary Tube Heating on Aflatoxin B1 Reduction

In order to increase the heat transfer capillary heating method was applied. Constant amount of Aflatoxin B1 (182±2 ppb) were heated by capillary method directly in oil bath at 120°C for 30-60-90 minutes, 160°C for 30-60 minutes and 200°C for 5 minutes. The aim of these experiments was to analyze the effect of peanut meal matrix on the Aflatoxin B1 detoxification. The results of the capillary tube heating are presented below in Figure 3.5 and 3.6.

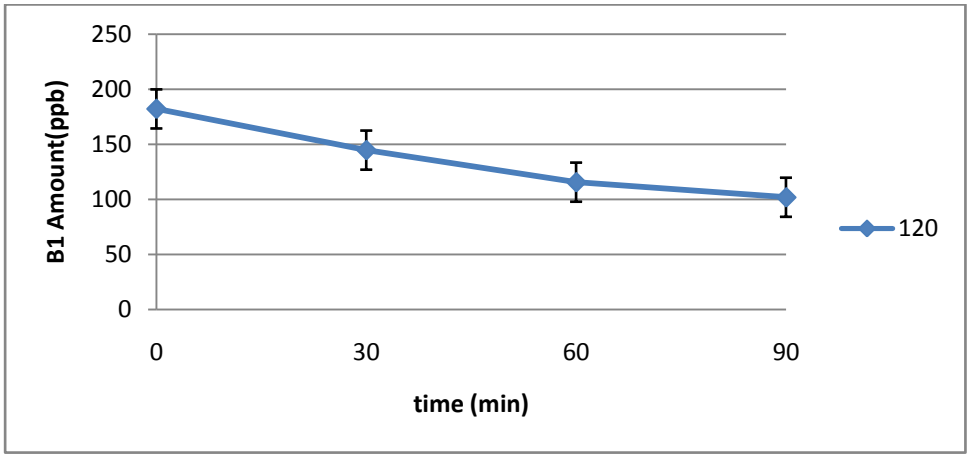


Figure 3. 5 Effect of Capillary Tube Heating at 120°C on Aflatoxin B1

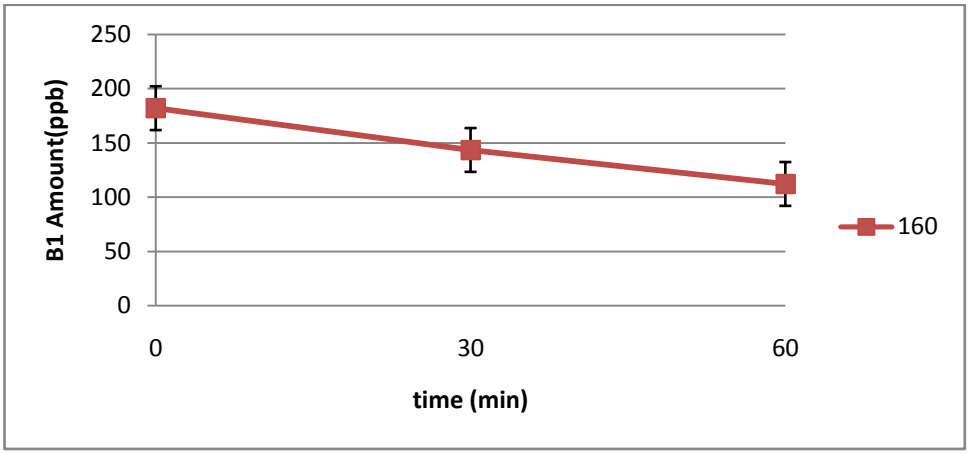


Figure 3. 6 Effect of Capillary Tube Heating at 160°C on Aflatoxin B1

When Aflatoxin B1 was heated in capillary tubes at 120°C for 90 minutes in temperature controlled oil bath, the Aflatoxin B1 level was decreased from 182.0 ppb to 101.8 ppb (Figure 3.5) on the other hand, when the same procedure was applied to the naturally Aflatoxin B1 contaminated peanut meal; level of Aflatoxin B1 was reduced only to 131.7 ppb (Figure 3.3). This shows the protective effect of the peanut meal matrix to heat inactivation of the Aflatoxin B1. The reason for such observation can be the solid structure of the sample material and also low thermal conductivity (0.13 W/m°C) of the peanut meal (Bitraa, et.al, 2010). Same case is valid for the process at 160°C for 60 minutes. When Aflatoxin B1 was directly heated in capillary tubes at 160°C for 60 minutes, the detoxification level was from 182.0 ppb to 112.2 ppb (Figure 3.6). However; when peanut meal is heated at the same conditions; Aflatoxin B1 level reduced to 136.8 ppb (Figure 3.4).

In addition to the solid structure of the peanut meal, heating mechanism in normal roasting and capillary tube heating was also different. In both roasting and capillary tube heating, cylindrical coordinates (r,Θ,z) can be selected for heat transfer mechanism. In roasting conditions; with the assumption of perfect mixing and no cold point; it can be considered that, heat transfer was in r and z direction (L:58 mm,

r: 23 mm). However in the case of capillary tube heating; L>> r (L:70 mm, r: 0.49 mm), heat transfer in r direction can be neglected, and it can be assumed that only heat transfer is in the z- direction. The equation for the given cases are given in the below figure. For the conditions in roasting case; the given thermal energy was transferred both in r and z- direction, however in the case of capillary heating; the given energy is directly transferred in z- direction which cause more uniform heating in the system.

$$\rho C_p \frac{\partial T}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}(kr\frac{\partial T}{\partial r}) + \frac{1}{r^2}\frac{\partial}{\partial \phi}(k\frac{\partial T}{\partial \phi}) + \frac{\partial}{\partial z}(k\frac{\partial T}{\partial z}) + g$$

Equation 1. Heat Transfer Equation in Cylindrical Coordinates

For roasting, the equation became in the following form:

$$\rho C_p \frac{\partial T}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}(kr\frac{\partial T}{\partial r}) + \frac{\partial}{\partial z}(k\frac{\partial T}{\partial z})$$

Equation 2. Modified Heat Transfer Equation in Cylindrical Coordinates for Roasting

However, in the case of capillary tube heating, the equation is only for the z-direction as given in the below equation:

$$\rho C_p \frac{\partial T}{\partial t} = \frac{\partial}{\partial z}(k\frac{\partial T}{\partial z})$$

Equation 3. Modified Heat Transfer Equation in Cylindrical Coordinates for Capillary Tube Heating

All the results are given in Appendix F.

3.4 Effect of Gamma Irradiation on Aflatoxin B1 in Peanut Meal

Naturally Aflatoxin B1 contaminated samples irradiated for10 kGy in TAEA. Results of the experiments are given in Figure 3.7.

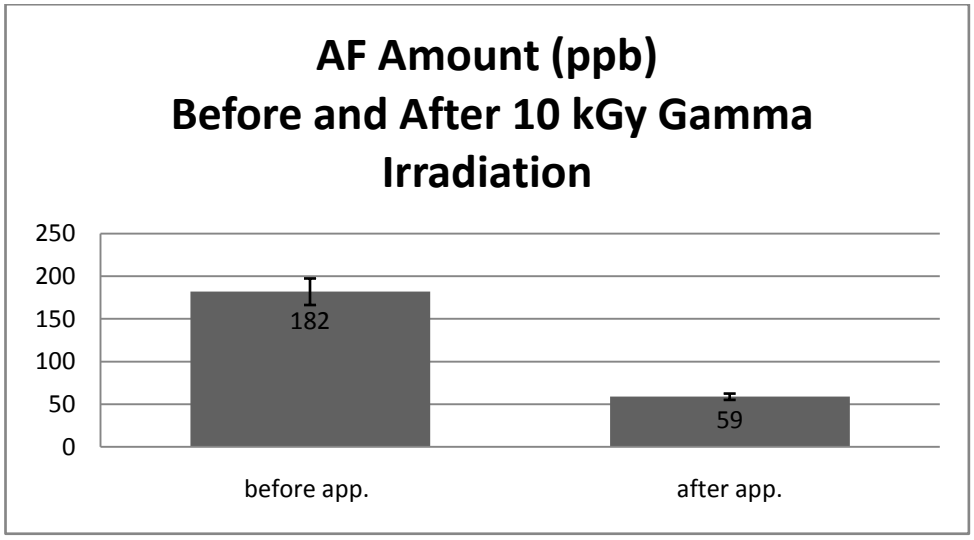


Figure 3. 7 Aflatoxin B1 Detoxification After 10 kGy Gamma Irradiation

Gamma irradiation resulted in maximum Aflatoxin B1 detoxification as 68% in peanut meal compared to heat applications carried out in our studies (Figure 3.7). Aflatoxin B1 level reductions by 75% to 100% by gamma rays irradiation of 1 kGy to 10 kGy respectively are reported in peanut initial Aflatoxin B1 level of 10 ppb (Van Dyck et.al, 1982). They claimed that; high levels of detoxification of the Aflatoxin B1 by irradiation were due to the presence of water that forms highly reactive free radicals because of the radiolysis. These radicals can react with the terminal furan ring and other double bonds of Aflatoxin B1 and give products of lower biological activities (Van Dyck, et.al, 1982). In our studies the Aflatoxin B1 level was reduced by 68% with 10 kGy gamma irradiation in 20% moisture content

peanut meal. The lower inactivation in our studies may be due to the initial Aflatoxin B1 levels of the sample and composition of the samples used in experiments.
The maximum reduction levels of the Aflatoxin B1 for different applications are illustrated in Figure 3.8.

Results given in Figure 3.8; maximum Aflatoxin B1 detoxification was attained at 200°C heat treatment for 5 minutes and also gamma irradiation at 10 kGy. As stated in the study of Rustom (1997); temperatures above 150°C are necessary for partial destruction of the toxin. The amount of destruction depends on the initial level of the contamination, type of toxin and food, heating time and temperature. It is obvious that; increasing temperature leads to higher increase in Aflatoxin B1 reduction. However over 200^0 C the time is the limiting factor for the sample stability.

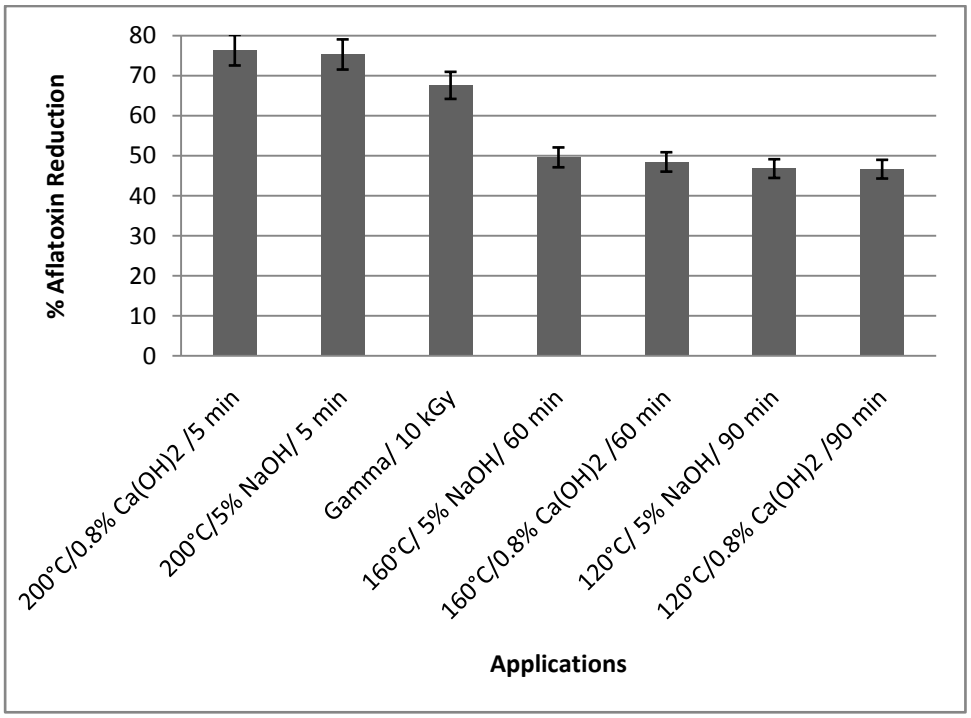


Figure 3. 8 Aflatoxin B1 Reduction Levels (%) in Peanut Meal for all Applications

3.5 Effect of Different Applications on Protein Content of Peanut Meal

The applied methods to decrease the Aflatoxin B1 may have detrimental effects on the protein content of the peanut meal. Effect of roasting and gamma irradiation on protein content of the peanut meal was analyzed by FTIR. Regardless of the state of sample (H_2O based or dry based environments), requiring less time and less sample amount, protein spectra can be obtained by FTIR and direct correlations between the IR amide I band frequencies and the secondary structure components of the proteins can be found. This is an advantage of FTIR to analyze the changes of protein's secondary and tertiary structure such as, alpha helices and beta sheets. In proteins, backbone structure was the important parameter for Amide I in which several internal coordinates contribute and this is determined by the secondary structure adopted by the polypeptide chain, reflecting the backbone conformation and hydrogen-bonding pattern. This determines the spectral parameters for absorbance band and also side chains (Kong & Yu, 2007). The absorbance intervals are given in Figure 1.6. Results for the peanut meal FTIR analysis are given in Figure 3.9 and 3.10.

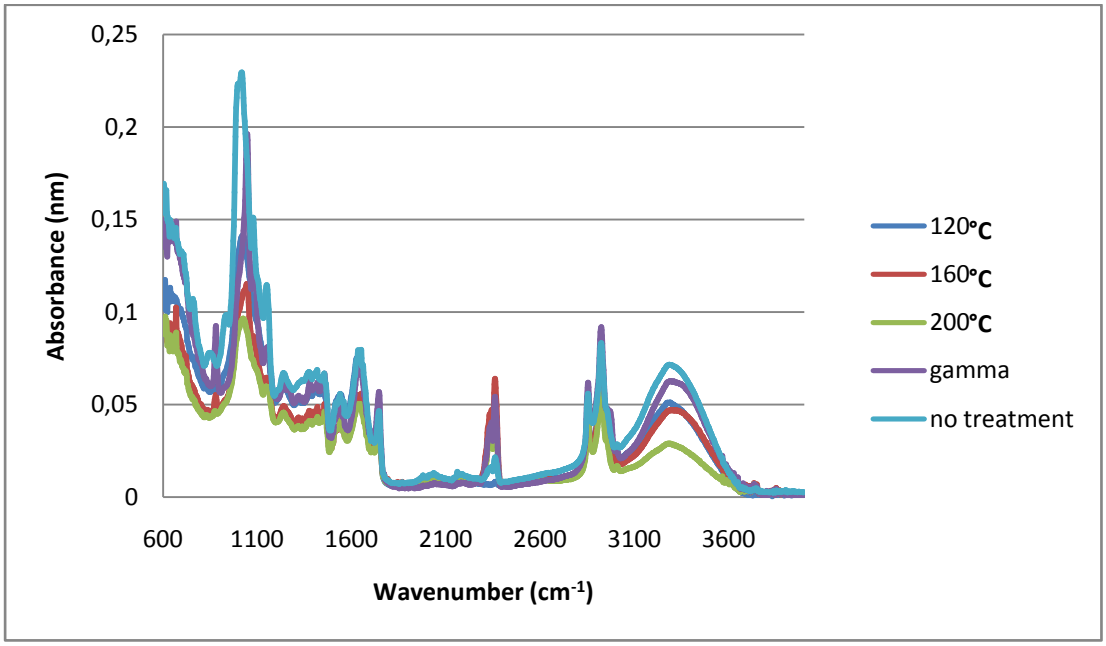


Figure 3.9 Protein Analyses by FTIR in the range of 600-4000 cm^{-1} wavenumber range

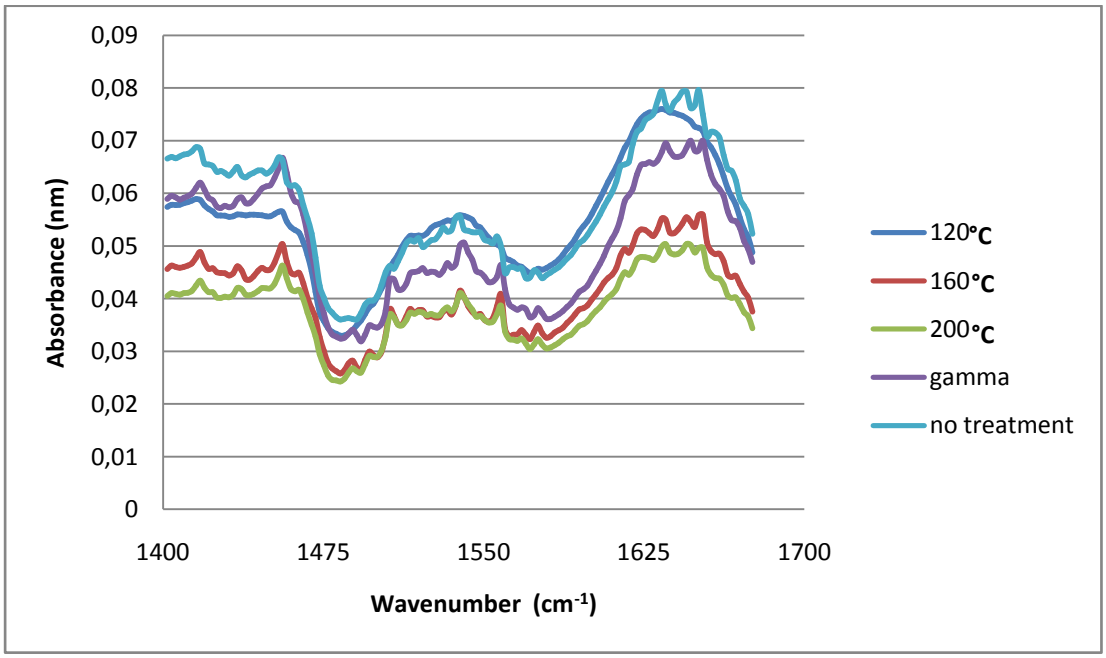


Figure 3.10 Protein Analyses by FTIR in the range of 1400-1600cm^{-1} wavenumber range

As mentioned in the study of Gallagher at 2014, characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II which are in the region of 1480-1690 cm^{-1}. Amide I region represents the C=O stretching mechanism in the wavenumber range of 1600-1690 cm^{-1} and Amide II region represents CN stretching and NH bending mechanism in the wavenumber range of 1480-1575 cm^{-1}.Figures 3.9 and 3.10 indicate protein contents of the samples treated with roasting at different temperatures and gamma irradiation also. In Figure 3.9; graph represents the overall change in the absorbance with respect to wavenumber range. In Figure 3.10, more detailed information can be obtained about the variance of amino acid structure and amount. As can be seen from the figure; the absorbance level of non- treated peanut meal were higher than that of other treated samples which means that C=O, C-N and N-H bonds in the amino acid structure of the peanut were not varied or denatured, in other words remain as its original form as background. However; higher temperature roasting (160°C and 200°C) cause significant reduction in the absorbance level of C=O, C-N and N-H bonds, which means that they were broken during roasting. In addition to that; the minimum reduction was observed in the case 120°C roasting and gamma irradiation. The reason for such a case is the lower thermal destruction of protein structure during gamma irradiation and lower thermal treatment.

3.6 Effect of Different Applications on the Structural Changes of Aflatoxin B1

To analyze the structural changes of Aflatoxin B1 by FTIR spectroscopy, it is essential to obtain a calibration curve for IR band intensity and Aflatoxin B1 content. One of the most important strengths of FTIR is the use of spectral rationing to discern small differences that would otherwise be missed in raw spectrum. Aflatoxin exhibits characteristic absorption bands at; 1440-1500 cm-1 for H-C-H bending, 1200- 1300 cm^{-1} for C-O, 1720-1745 cm^{-1} for C=O ad 1450- 1500 cm^{-1} for C=C.

In Figures 3.11 and 3.12; C-O and H-C-H changes in the Aflatoxin B1 structure was given. According to the literature; these changes were due to the cleavage of methyl group attached to the central phenyl ring of Aflatoxin B1. However this cleavage does not have an important effect on the toxicity of the Aflatoxin B1 (Proctor, et.al, 2004).

Aflatoxin B1 toxicity was reported to be changed by the opening of terminal furan ring and lactone ring (Proctor, et.al, 2004). The main toxicity potential of Aflatoxin B1 and G1 is due to the double bond in the terminal furan ring, in addition to that; in Aflatoxin G1 and G2; there are 2 lactone ring structure which makes them more susceptible for detoxification.

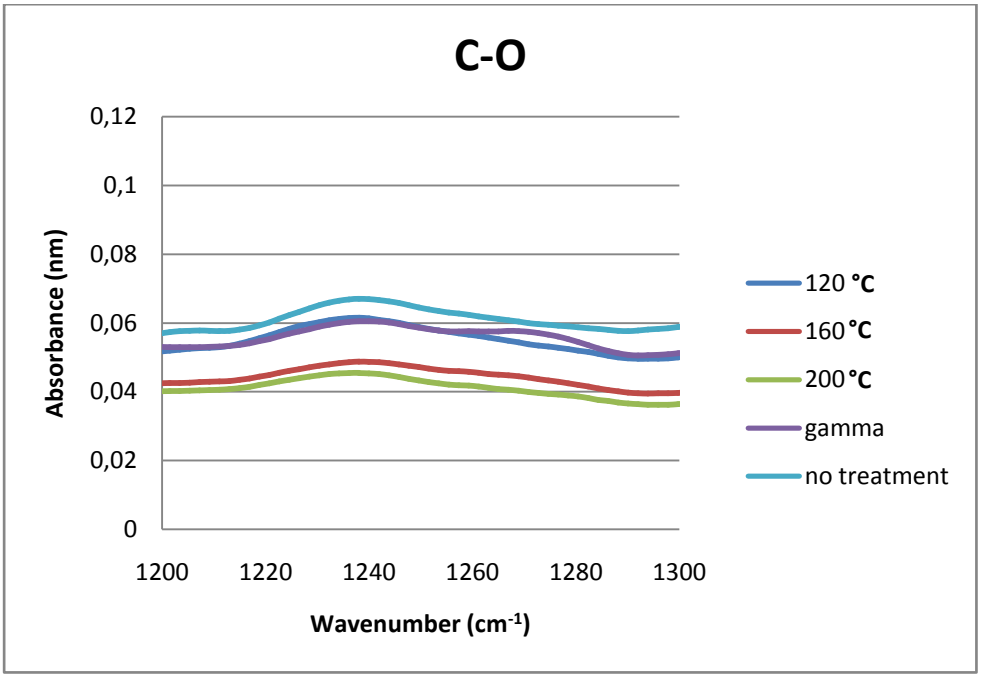


Figure 3. 11 Change in C-O Bonds in Aflatoxin B1 Structure

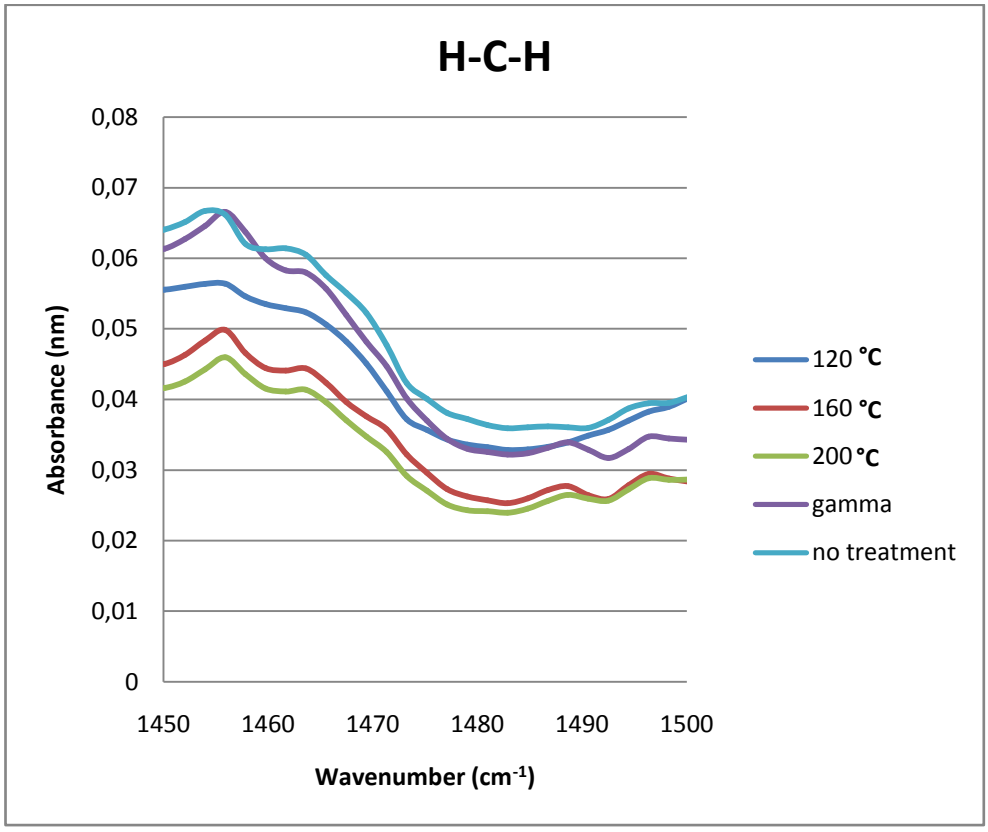


Figure 3. 12 Change in H-C-H Bonds in Aflatoxin B1 Structure

In Figures 3.14 and 3.15; the difference in the level of C=C and C=O bonds represents the opening of terminal furan ring and opening of lactone ring in Aflatoxin B1 (Figure 3.13) (Mirghani, et.al, 2001).

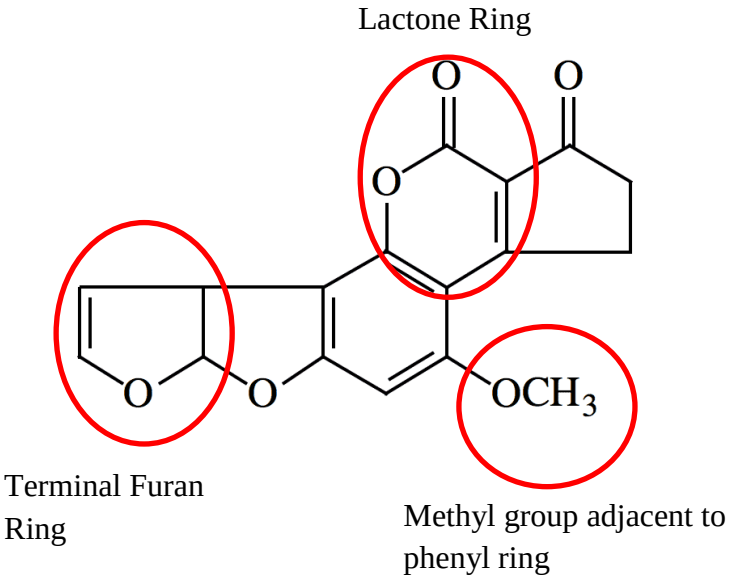


Figure 3.13 Possible Detoxification Points of Aflatoxin B1

In the presence of moisture in the samples; it is possible to obtain opening of more furan and lactone rings because of the thermolysis of the water molecules during heating (Kaya-Çeliker, et.al, 2011). During thermolysis of water, free OH^- and H^+ groups bind to the double bonds in the furan ring and also cause opening of lactone ring and form carboxylic group. This structural changes are observed as the decrease of C=C bonds peaks because of opening of ring structures and increase of C=O bonds due to the formation of carboxylic group. On the other hand; in the case of gamma irradiation; because of the high potential of gamma irradiation to form free radicals; the amount of random cleavages were increased, and this was the main reason of the differences in C=O bond peak increase. In general; it can be observed that; in the presence of water molecules; high heat treatment cause more opening of furan and lactone rings. On the other hand; gamma irradiation may cause additional cleavages of double bonds in Aflatoxin B1 structure.

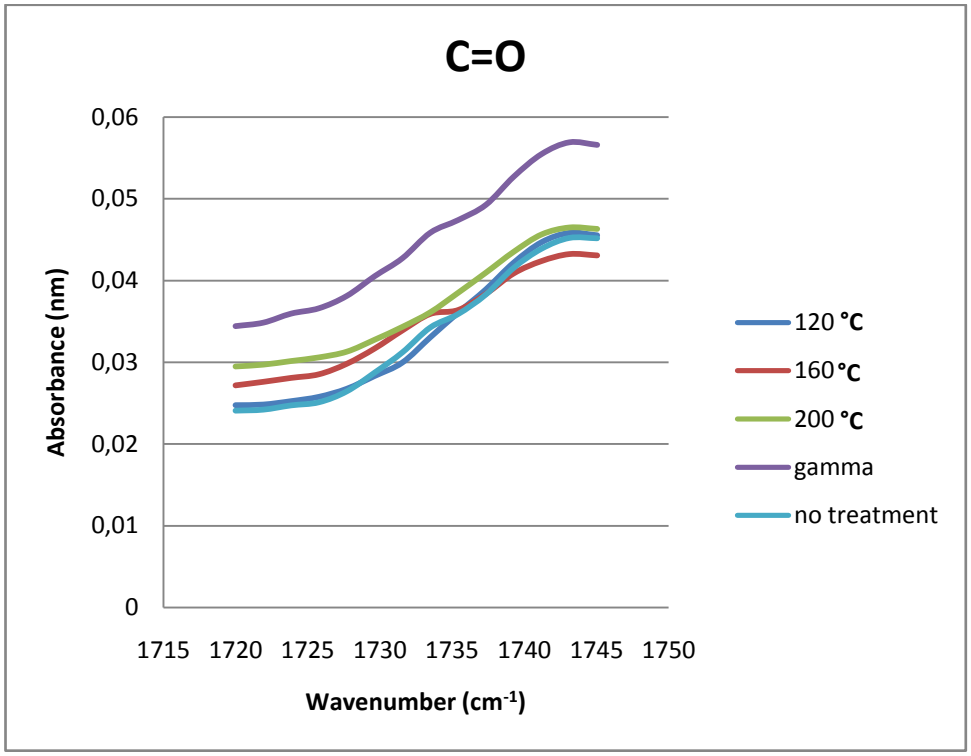


Figure 3. 14 Change in C=O Bonds in Aflatoxin B1 Structure

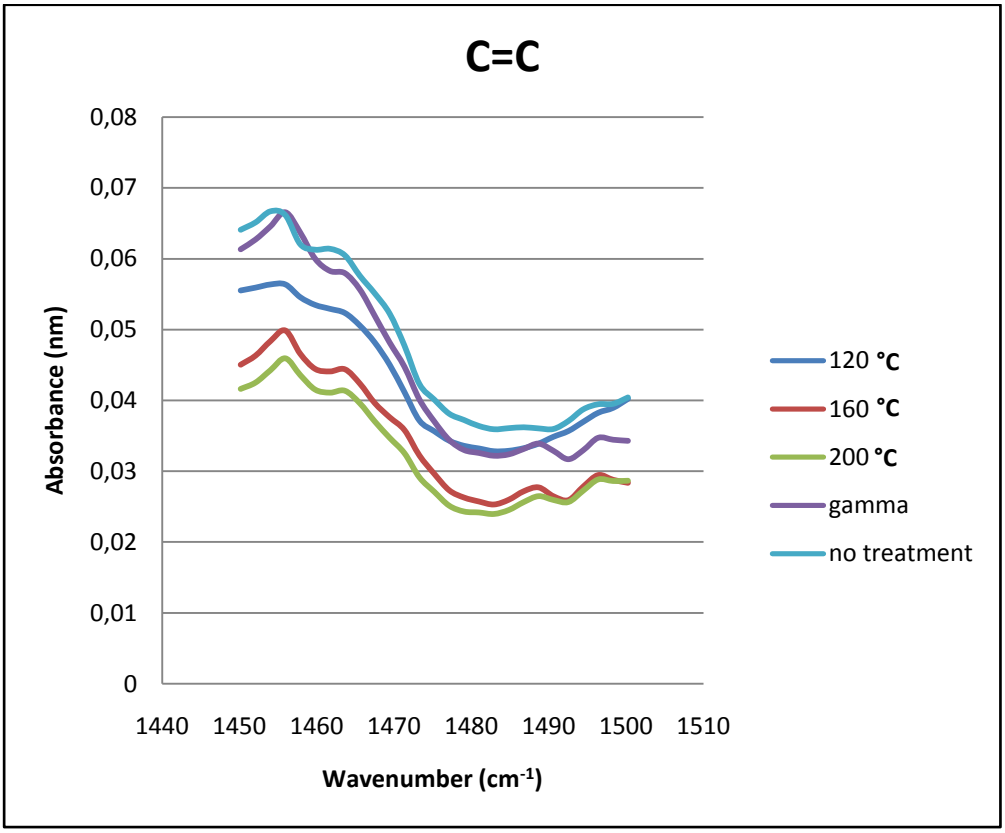


Figure 3. 15 Change of C=C Bonds in Aflatoxin B1 Structure

3.7 Effect of Roasting and Gamma Irradiation on Toxicity of Aflatoxin B1

Glutathione peroxidase is a type of liver enzyme with the main role of protecting the organism from oxidation. The main reaction of Glutathione Peroxidase (Figure 3.16) is:

"$2GSH + H_2O_2 \rightarrow GS\text{-}SG + 2H_2O$

where reduced monomeric glutathione refers to GSH, and GS-SG represents glutathione disulfide. Glutathione reductase (GR) then reduces the oxidized glutathione to complete the cycle:

GS-SG + NADPH + $H^+ \rightarrow$ 2 GSH + $NADP^+$ "((Martino & Gallo, 2009).

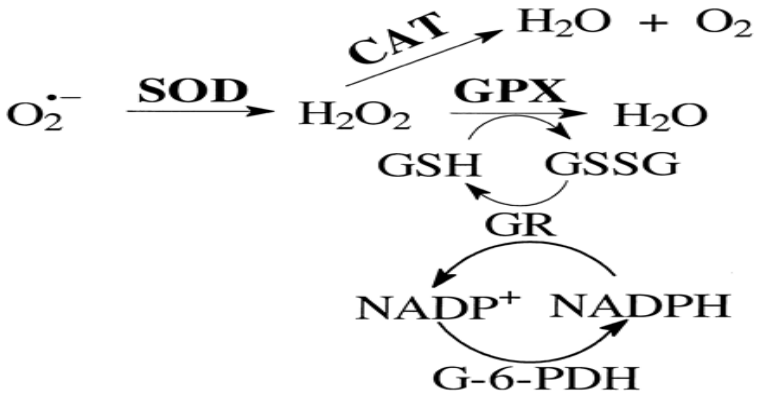


Figure 3. 16 Representation of Antioxidant Role of GPx

In order to analyze the effect of roasting and gamma irradiation on toxicity of aflatoxin B1, glutathione peroxidase was used as the target enzyme.

During experiments; GPx activity was studied by spectrophotometric analyses for 60 seconds at 37°C, wavelength of 340 nm. The activity of enzyme without addition of Aflatoxin B1, with the addition of 100 ppb Aflatoxin B1, with the addition of 120°C/30 minutes treated, 120°C/60 minutes treated, 120/°C/90 minutes treated, 160°C/30 minutes treated, 160°C/60 minutes treated, 200°C/5 minutes treated and gamma irradiated Aflatoxin B1 were measured.

As stated in the study of Devendran and Balasubramanian (2011); Aflatoxin B1 treatment causes significant reduction in the activities of glutathione peroxidase and reductase in the liver and kidney of rats. According to our studies glutathione peroxidase activity was dramatically affected by non-treated Aflatoxin B1 and

Aflatoxin B1 heated at 120°C/30 minutes samples (Figure 3.17). The minimum activity reduction was seen for Aflatoxin B1 heated at 200°C/5 minutes and gamma irradiation treatments. The reduction of glutathione peroxidase activity with the samples correlates with the reduction of Aflatoxin B1 levels by the treatments applied (Fig. 3.8).

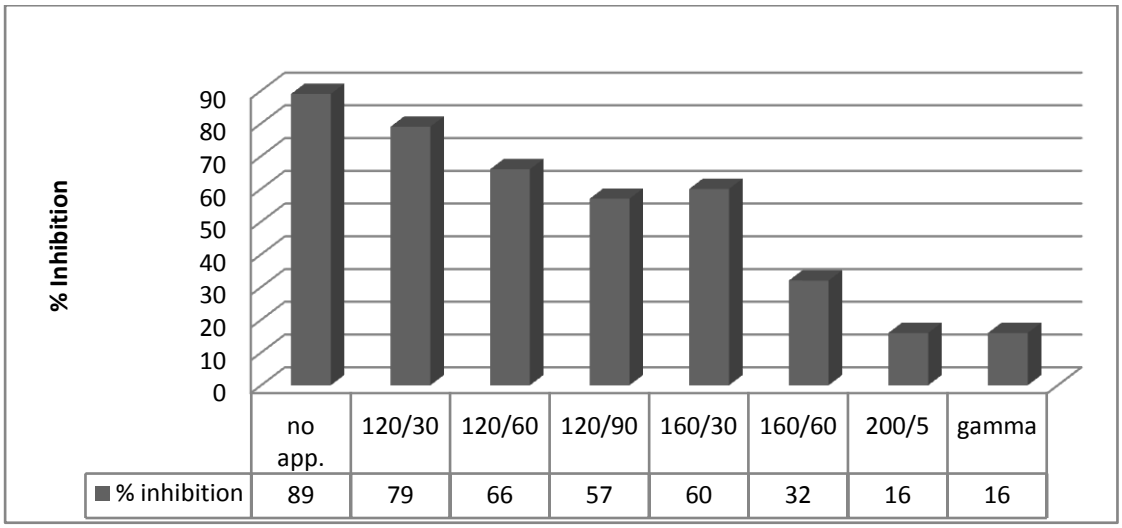


Figure 3. 17 Percent Inhibition of Glutathione Peroxidase

According to the results; it can be claimed that applied treatments did not increase the toxicity of Aflatoxin B1 on liver antioxidant enzymes or produced by products with toxic activity. Change in the structure of Aflatoxin B1 due to treatments did not have an additional toxic effect on liver antioxidant enzymes that shows applied methods in this study do not have any increase effect on the toxicity of the Aflatoxin B1.

3.7.1 Effect of Different Applications on Glutathione Peroxidase Activity

The Glutathione Peroxidase (GPx) activities were determined by spectrophotometric analyses. During analyses; 5, 10 and 50 µL of Glutathione was used as substrate. According to the analyses; enzyme kinetic activities were fitted to the Lineweaver-Burk model. In Figure 3.18, linearized form of 1/substrate vs. 1/enzyme velocity graph is given.

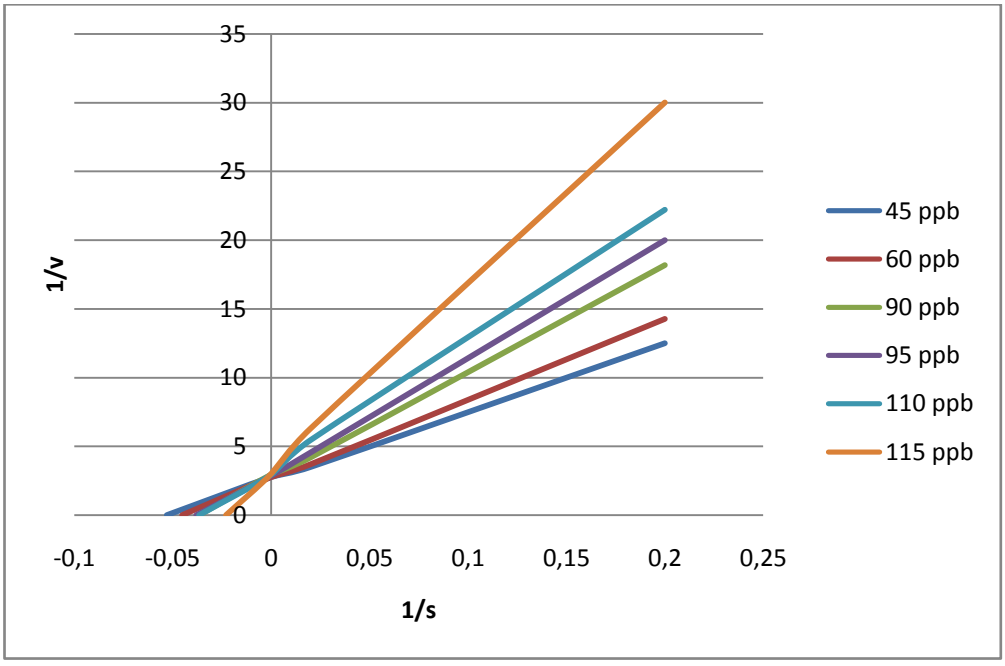


Figure 3.18 Lineweaver-Burk Plotting for different Aflatoxin B1 concentrations

Although there is no study about inhibition of glutathione peroxidase by Aflatoxin B1; according to the kinetic analyses given in Figure 3.18, constant V_{max} (0.35± 0.01) and changing K_m (25.73± 9.53) values were observed. According to these analyses, it can be assumed the inhibitory mechanism of Aflatoxin B1 on glutathione peroxidase as competitive inhibition.

Glutathione peroxidase's contact regions consist of 16 amino acids which are non-polar and polar zones. Its' catalytically active center contains selenocysteine which makes the substrate binding easier and increase the reaction rate. The catalytically active selenocysteine residues could be located at the N-terminal ends of α-helices forming substructures together with two adjacent parallel β- strands. According to the study of Epp in 1983; it was observed that; substrate binds to the enzyme by Hydrogen active side (Epp, et.al, 1983). According to the same study; active side of the substrate is observed as an hydrogen donor, and selenoenzyme (Glutathione peroxidase) binds this hydrogen donor part of the substrate.

The principle mechanism for competitive enzyme inhibition is the binding of inhibitory toxin to the active side of the enzyme and reduces the affinity of enzyme for binding to the substrate. According to the studies of Ren (1997), active side of the glutathione peroxidase binds to the hydrogen of the substrate (Ren, et.al, 1997). For the case of aflatoxin inhibition, Aflatoxin B1 was supposed to bind to the active side of the glutathione peroxidase by giving hydrogen like glutathione. So, it is logical to assume that, aflatoxin bind the active side of the enzyme through methyl group adjacent to the central phenyl ring instead of cyclic structures which presents large structures. During detoxification processes of Aflatoxin B1, because of separation of the methyl group from the Aflatoxin B1 structure, and decrease in the amount of H-C-H bonds observed in FTIR analyses, affinity of Aflatoxin B1 to the active side of glutathione peroxidase is eliminated. It is seen by the unreduced activities measured for the GPx in the presence of heat (120°C, 160°C, 200°C) and gamma irradiated (10 kGy) Aflatoxin B1.

CHAPTER 4

CONCLUSION

According to the results obtained from our experiments; higher heat treatments (above 120°C) and gamma irradiation (10 kGy) are effective on Aflatoxin B1 level reduction. Maximum Aflatoxin B1 detoxification was observed at 200°C/ 5 minutes roasting and gamma irradiation (10 kGy) with 77% and 68% reduction levels, respectively.

During experiments; the level of Aflatoxin B1 was not reduced to the legal levels set by authorities (20 ppb). The reason for such a case is the high level of initial contamination of Aflatoxin B1 in the peanut meal. The reduction levels may reach to regulated levels by increasing roasting temperature and roasting time or irradiation doses; however; these applications will lead to structural changes on peanut meal and its protein content.

During studies; roasting reduced the level of protein of peanut meal. Minimum protein denaturation was observed during gamma irradiation.

In addition to the detoxification levels, used treatments did not have an increasing effect on toxicity of Aflatoxin B1. These analyses were observed by glutathione peroxidase activity.

Combination of several treatments may be more effective on Aflatoxin B1 detoxification other than using single technique for further researches.

RECOMMENDATIONS

- Toxicity analyses should be performed on liver tissue cultures.
- Combination of several techniques may be more effective on Aflatoxin B1 reduction other than using single process.
- Decrease in protein levels may not be directly attributed to the loss of nutritive value, has to be studied by animal feeding studies.
- Structural changes resulting from applications has to be studied with advanced techniques.

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APPENDIX A

x-axis represents time (minutes) and y- axis represents absorbance (nm) in graphs given in Appendix A.

HPLC GRAPHS OF DRY ROASTED SAMPLES' AFLATOXIN ANALYSES

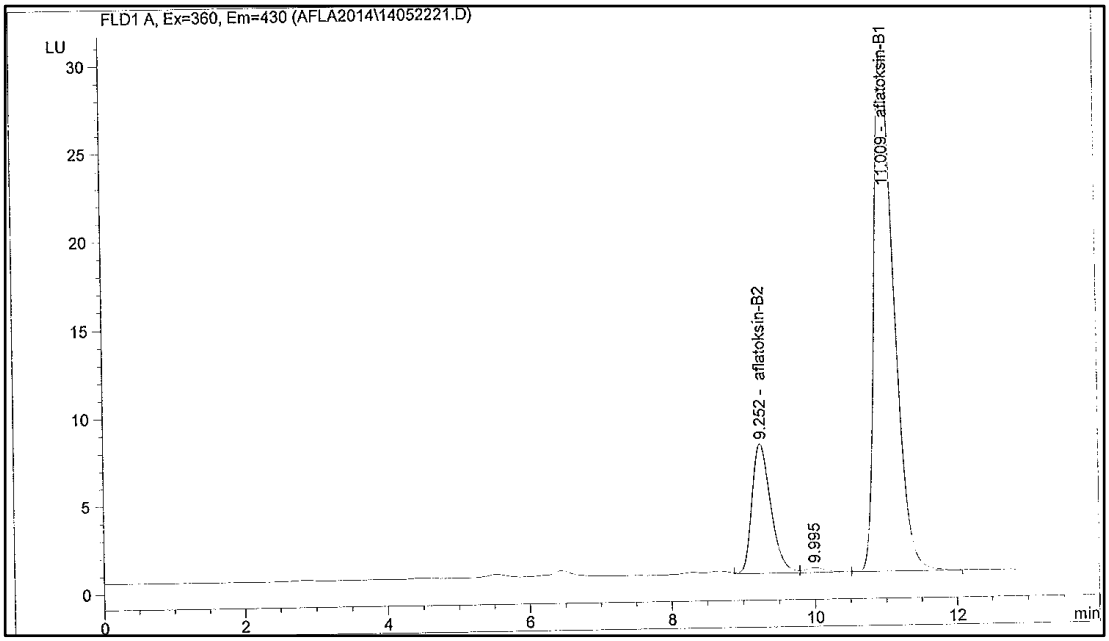

Figure A.1 Dry Roasting at 120°C for 30 minutes with no chemicals

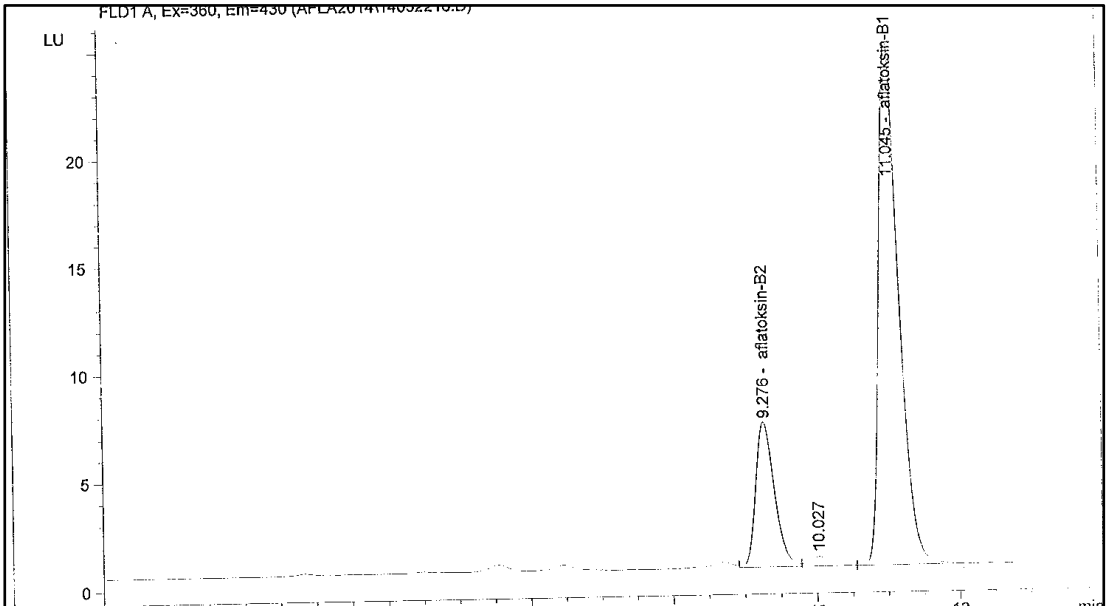

Figure A.2 Dry Roasting at 120°C for 60 minutes with no chemicals

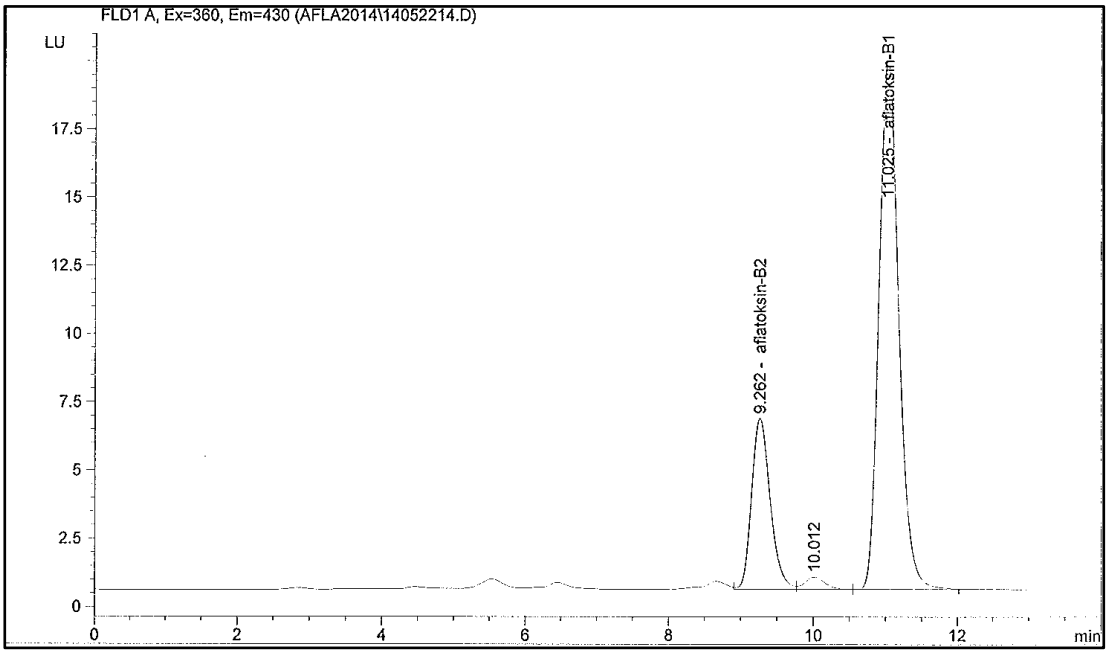

Figure A.3 Dry Roasting at 120°C for 90 minutes with no chemicals

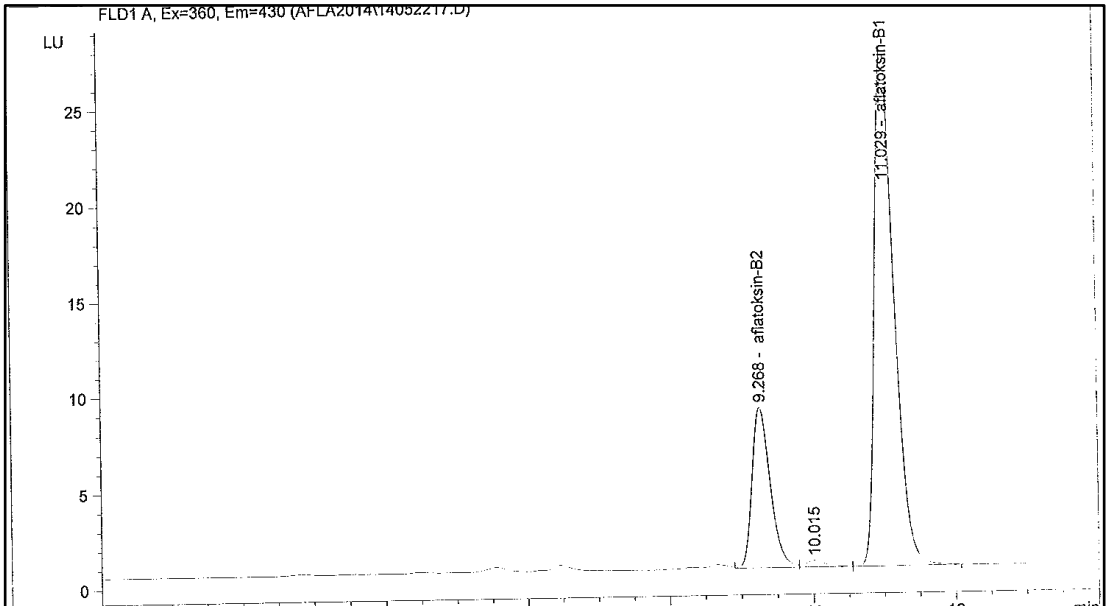

Figure A.4 Dry Roasting at 120°C for 30 minutes with the addition of 0.2% $Ca(OH)_2$

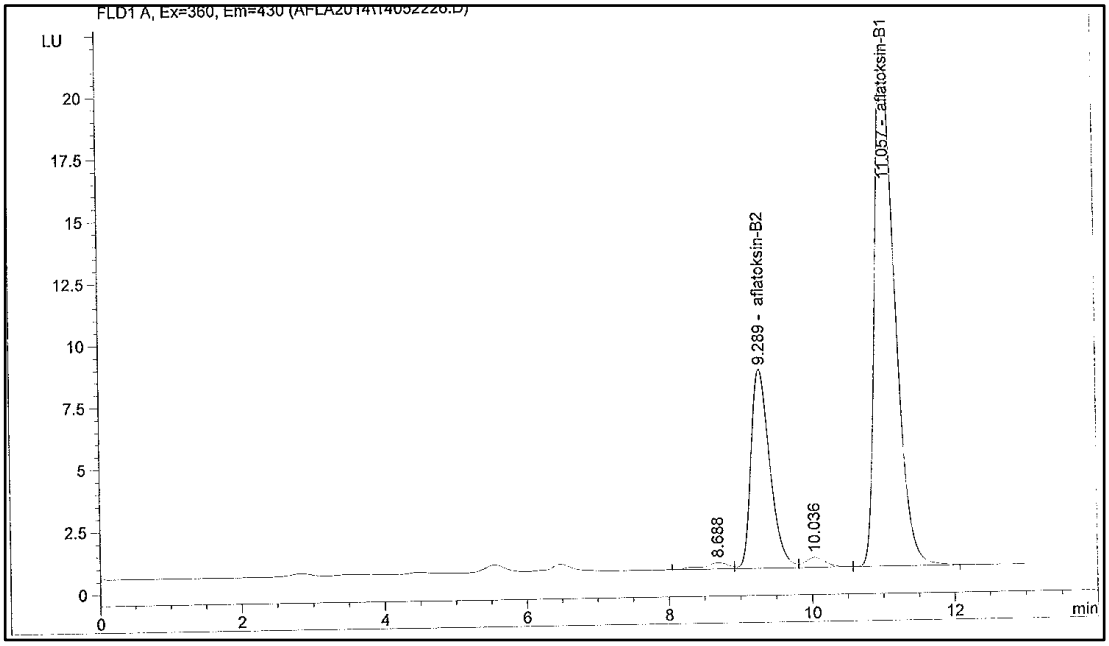

Figure A.5 Dry Roasting at 120°C for 60 minutes with the addition of 0.2% $Ca(OH)_2$

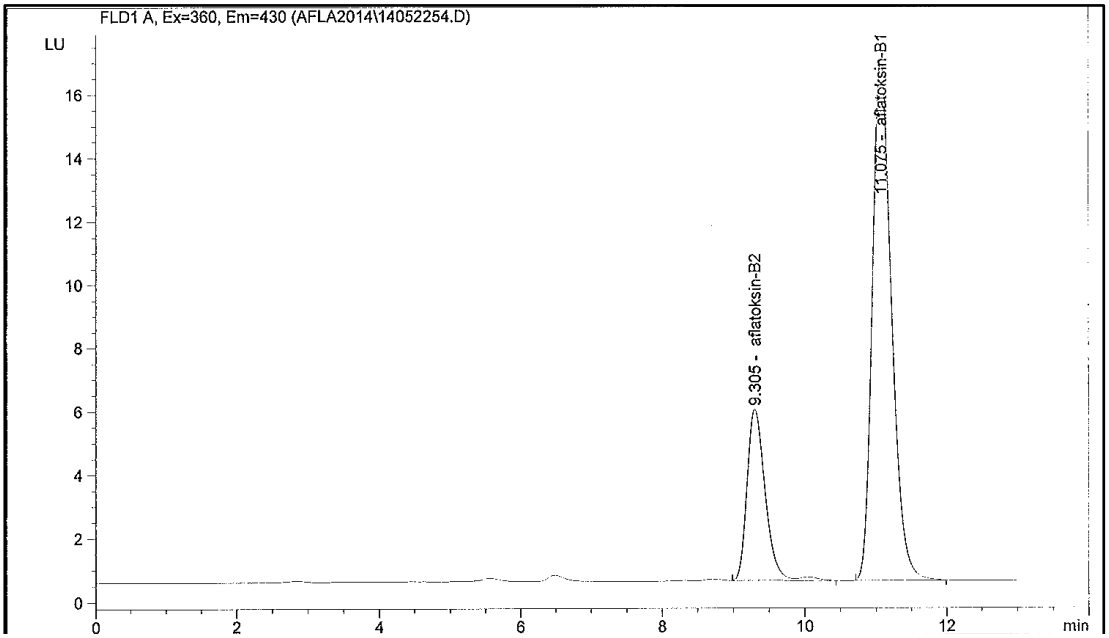

Figure A.6 Dry Roasting at 120°C for 90 minutes with the addition of 0.2 % $Ca(OH)_2$

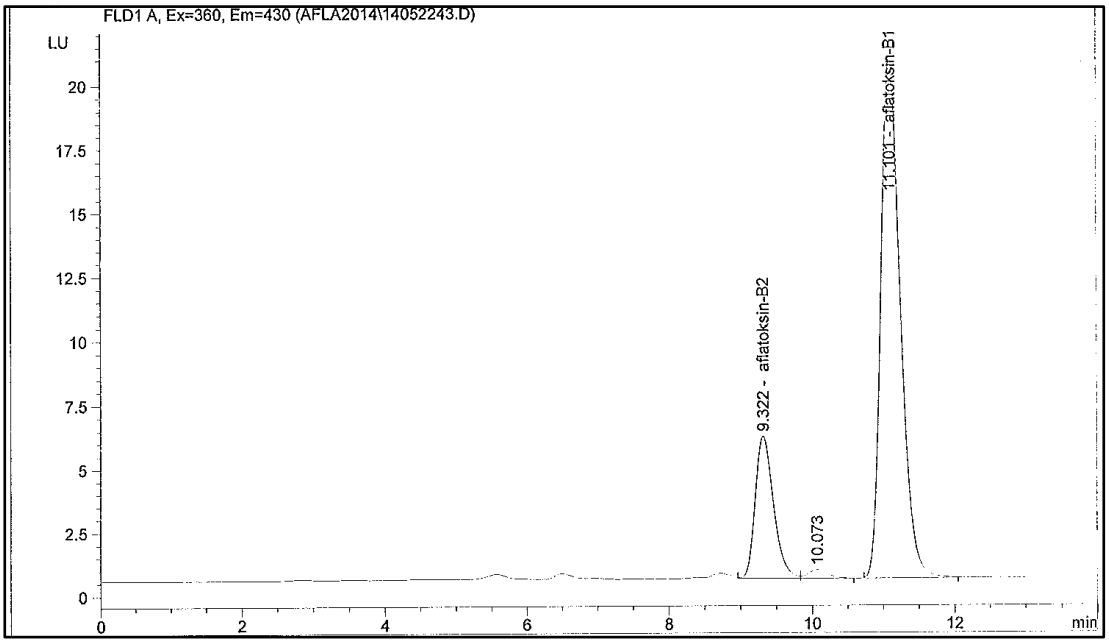

Figure A.7 Dry Roasting at 120°C for 30 minutes with the addition of 0.8% $Ca(OH)_2$

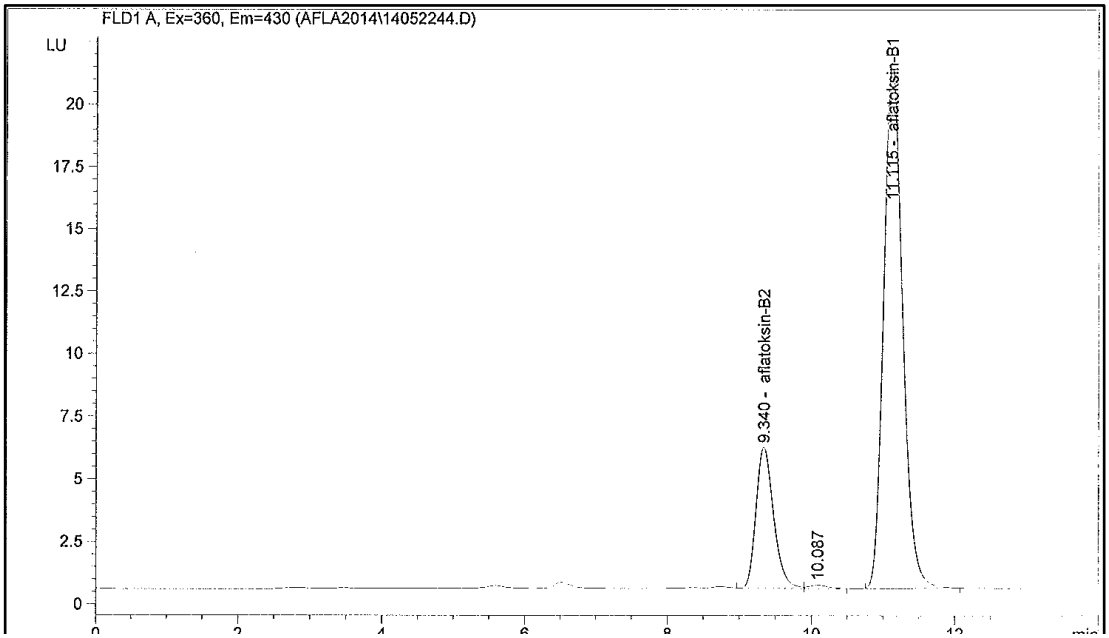

Figure A.8 Dry Roasting at 120°C for 60 minutes with the addition of 0.8% $Ca(OH)_2$

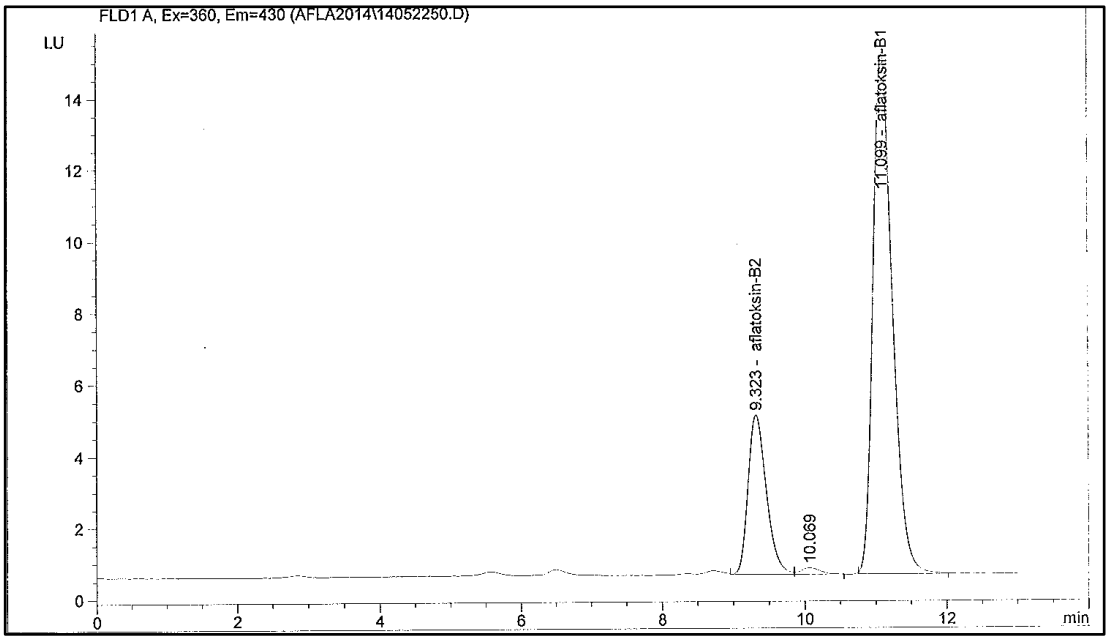

Figure A.9 Dry Roasting at 120°C for 90 minutes with the addition of 0.8% $Ca(OH)_2$

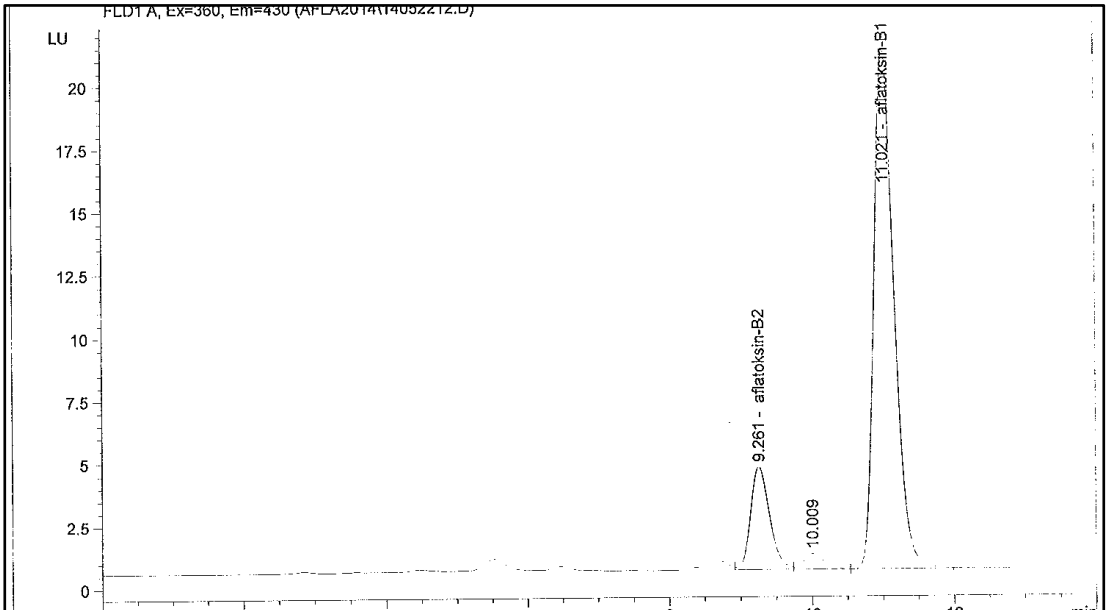

Figure A.10 Dry Roasting at 160°C for 30 minutes with no chemicals

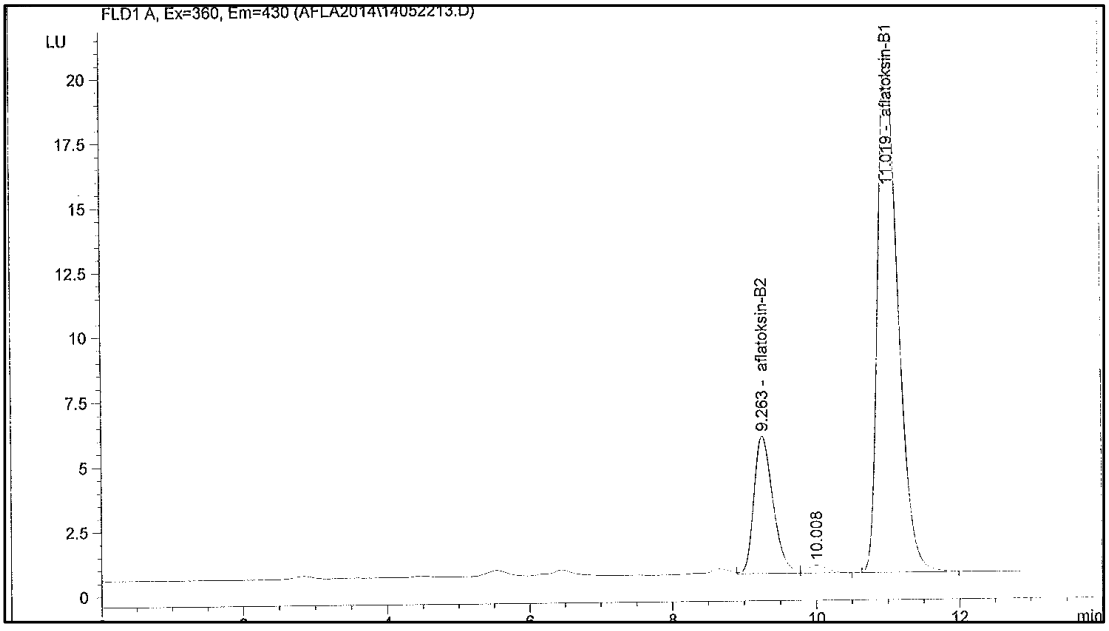

Figure A.11 Dry Roasting at 160°C for 60 minutes with no chemicals

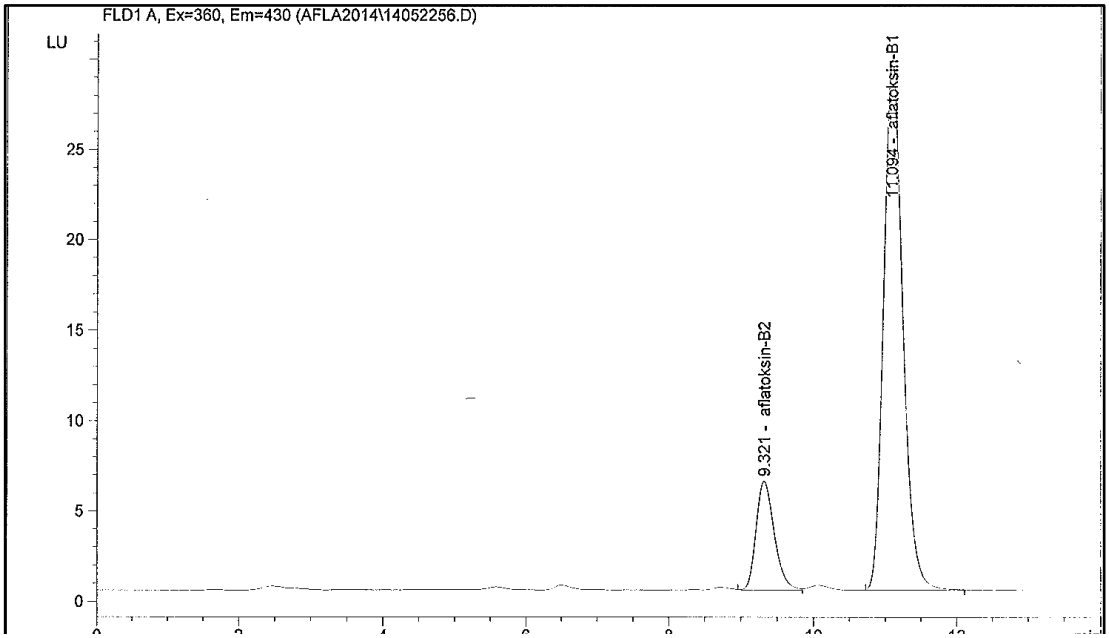

Figure A.12 Dry Roasting at 160°C for 30 minutes with the addition of 0.2% $Ca(OH)_2$

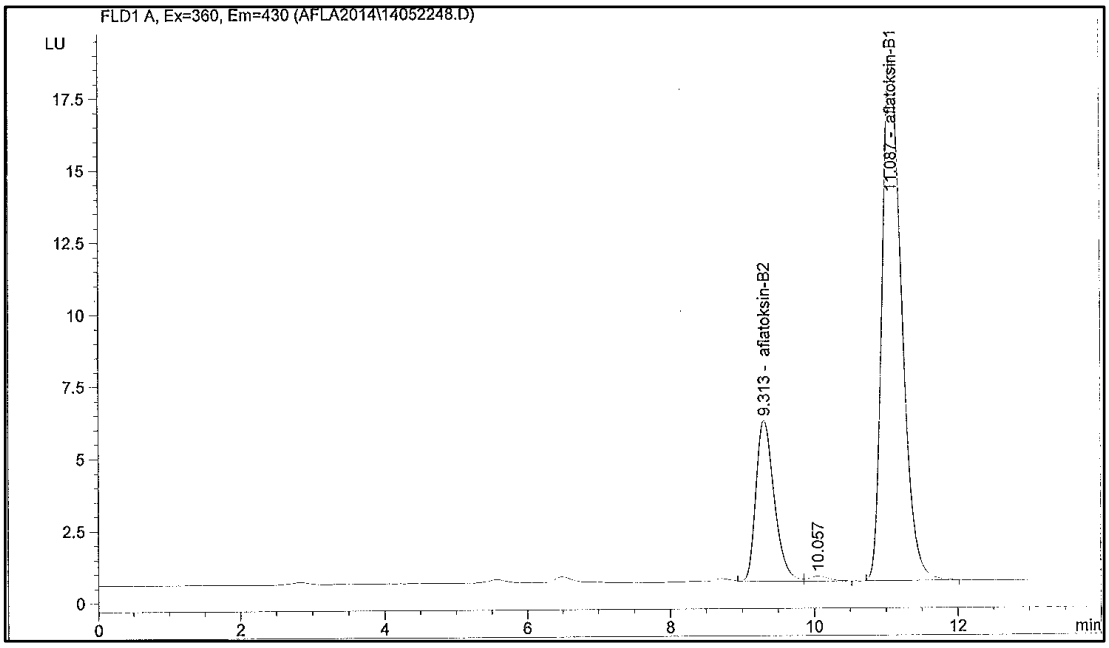

Figure A.13 Dry Roasting at 160°C for 60 minutes with the addition of 0.2% $Ca(OH)_2$

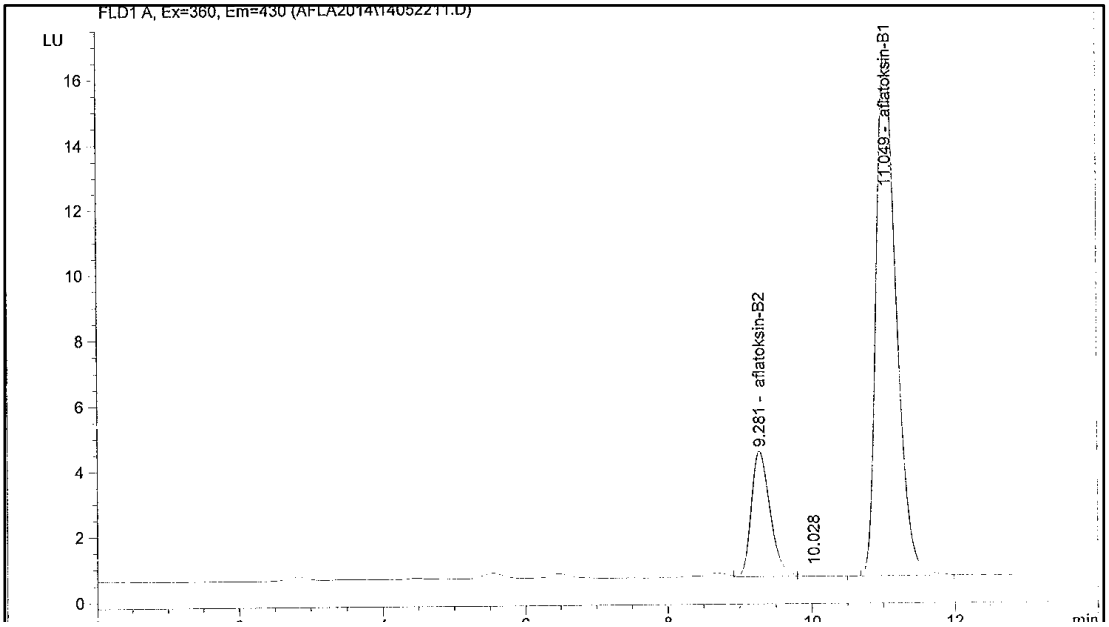

Figure A.14 Dry Roasting at 160°C for 30 minutes with the addition of 0. 8% $Ca(OH)_2$

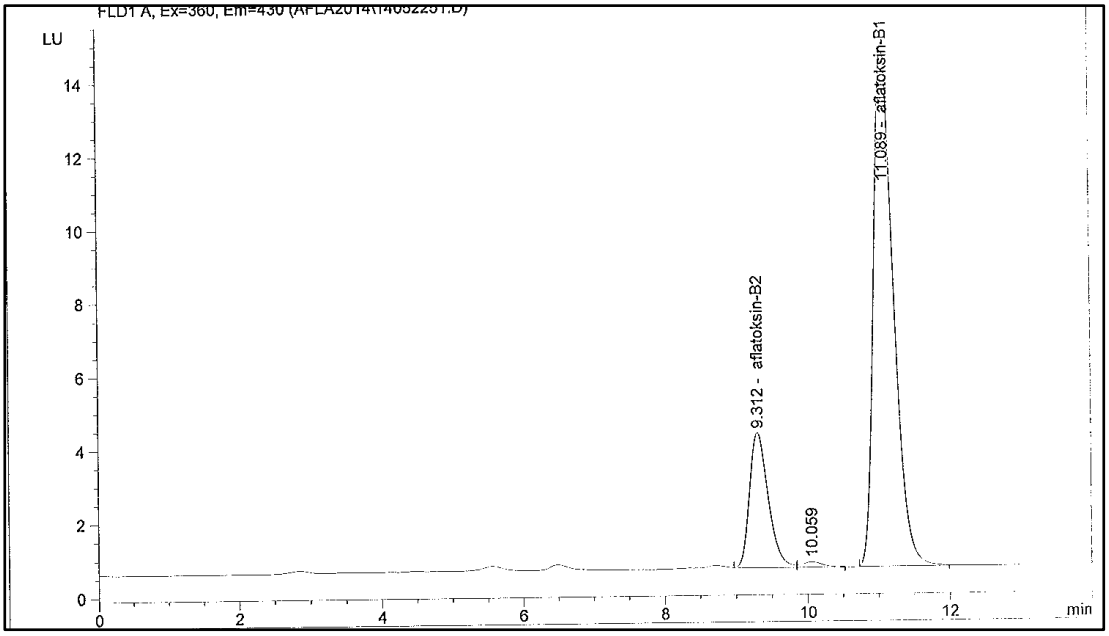

Figure A.15 Dry Roasting at 160°C for 60 minutes with the addition of 0.8 % $Ca(OH)_2$

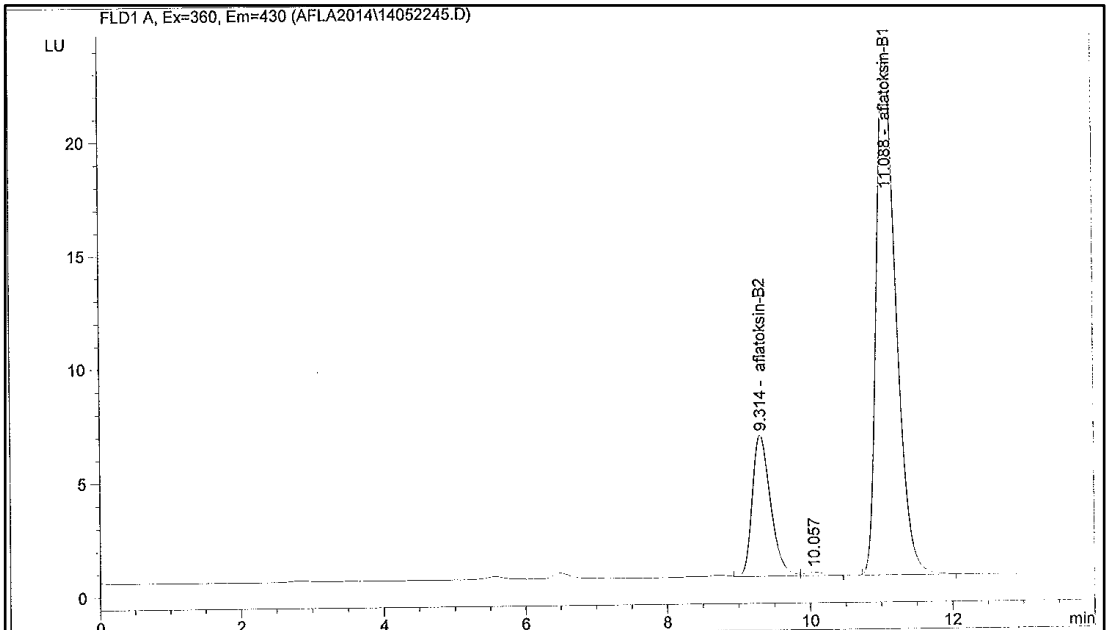

Figure A.16 Dry Roasting at 200°C for 5 minutes with no chemical addition

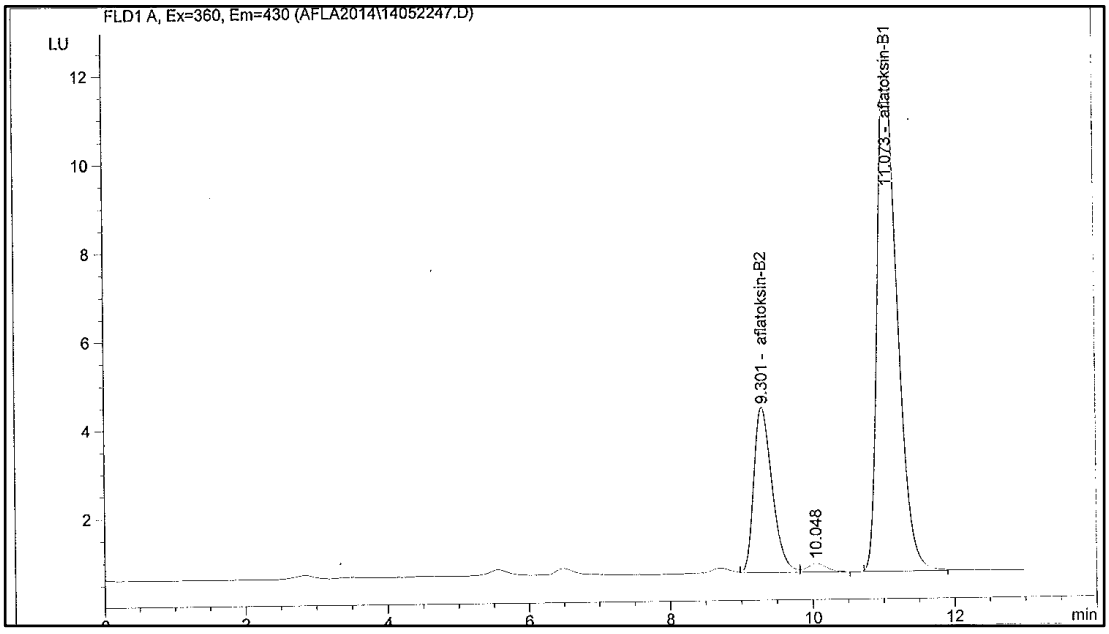

Figure A.17 Dry Roasting at 200°C for 5 minutes with the addition of 0.2% $Ca(OH)_2$

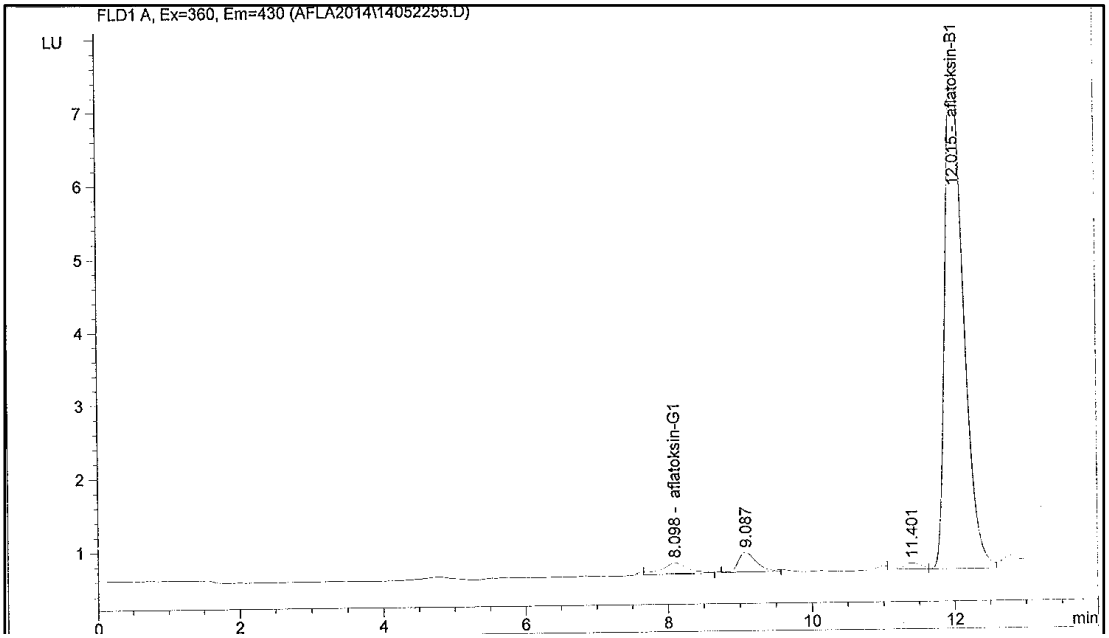

Figure A.18 Dry Roasting at 200°C for 5 minutes with the addition of 0.8% $Ca(OH)_2$

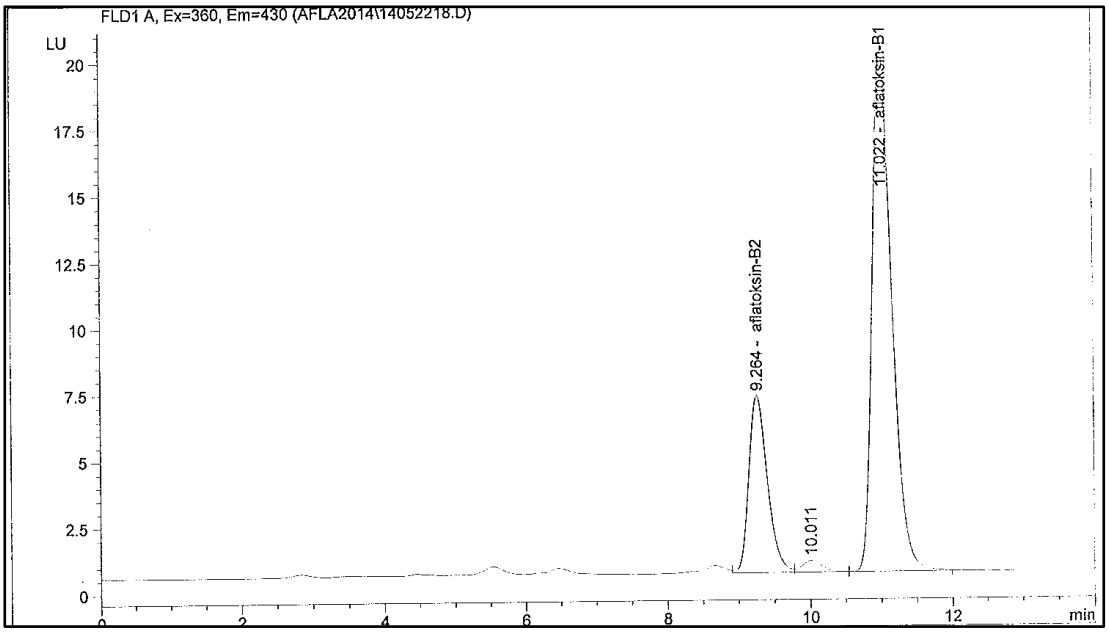

Figure A.19 Dry Roasting at 120°C for 30 minutes with the addition of 3% NaOH

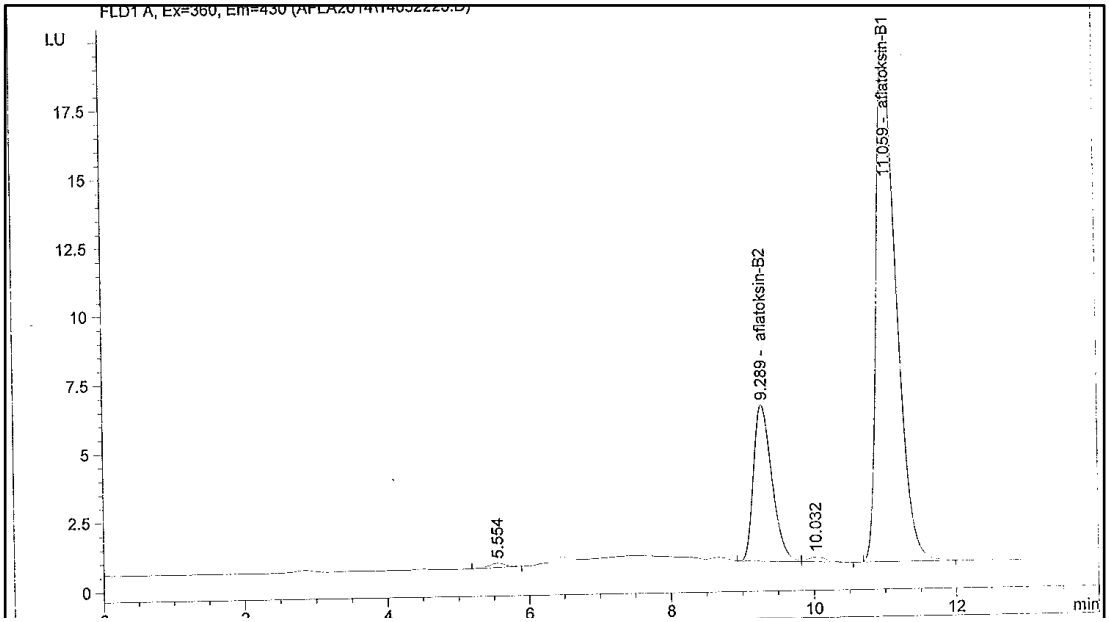

Figure A.20 Dry Roasting at 120°C for 60 minutes with the addition of 3% NaOH

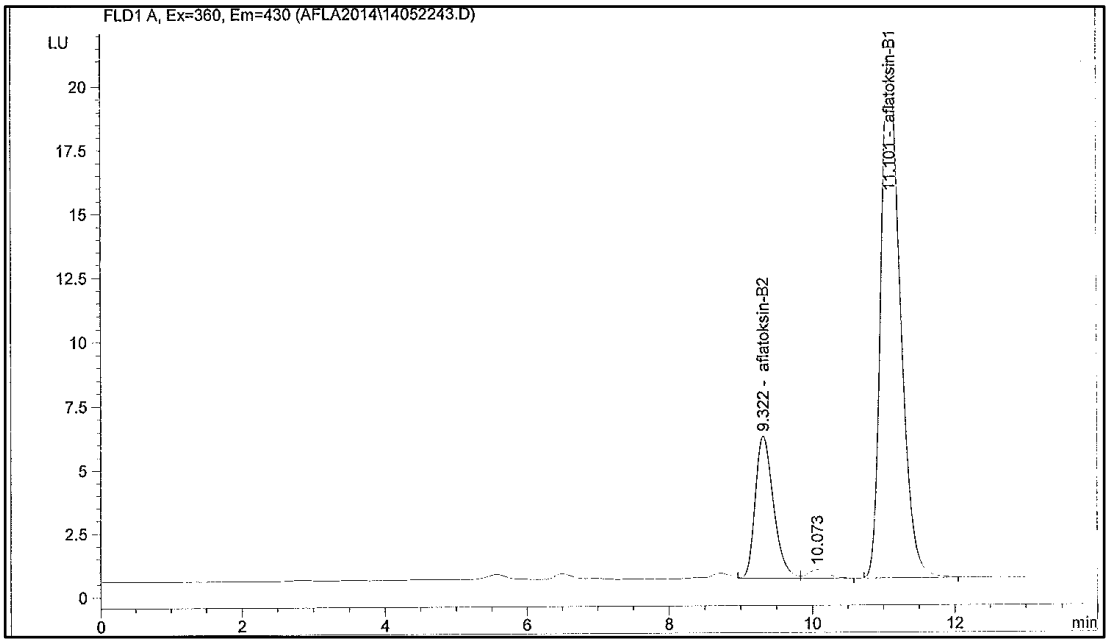

Figure A.21 Dry Roasting at 120°C for 90 minutes with the addition of 3% NaOH

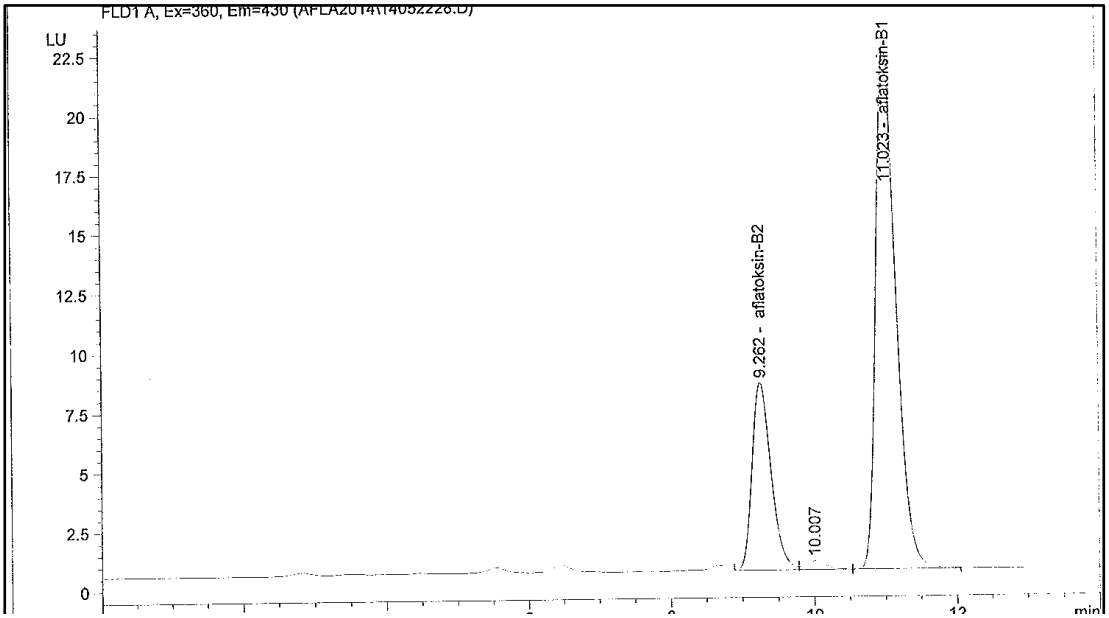

Figure A.22 Dry Roasting at 120°C for 30 minutes with the addition of 5% NaOH

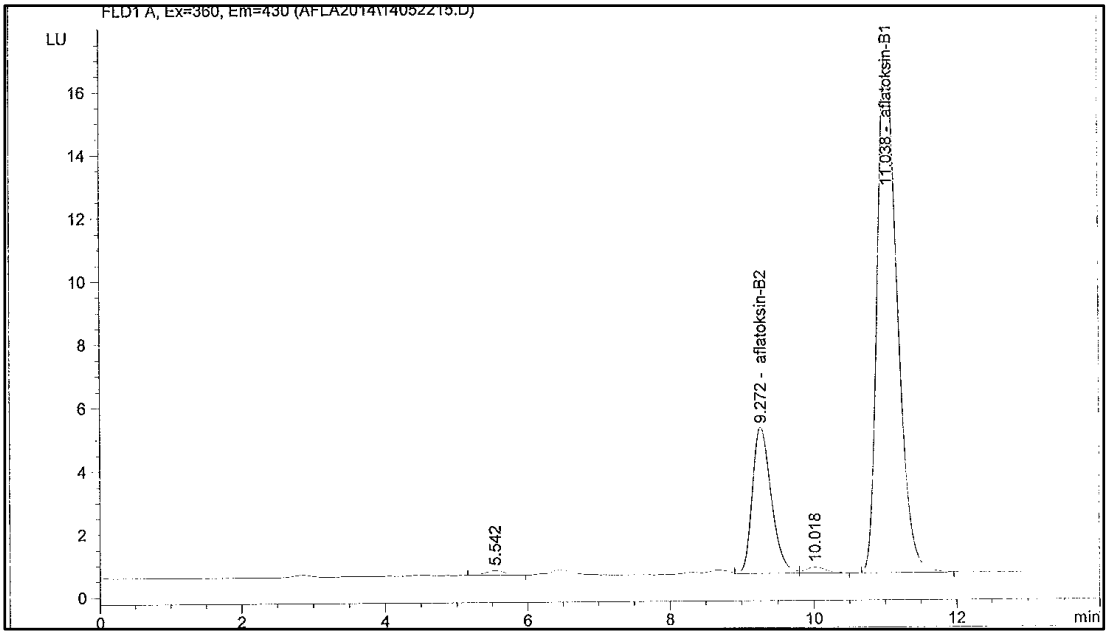

Figure A.23 Dry Roasting at 120°C for 60 minutes with the addition of 5% NaOH

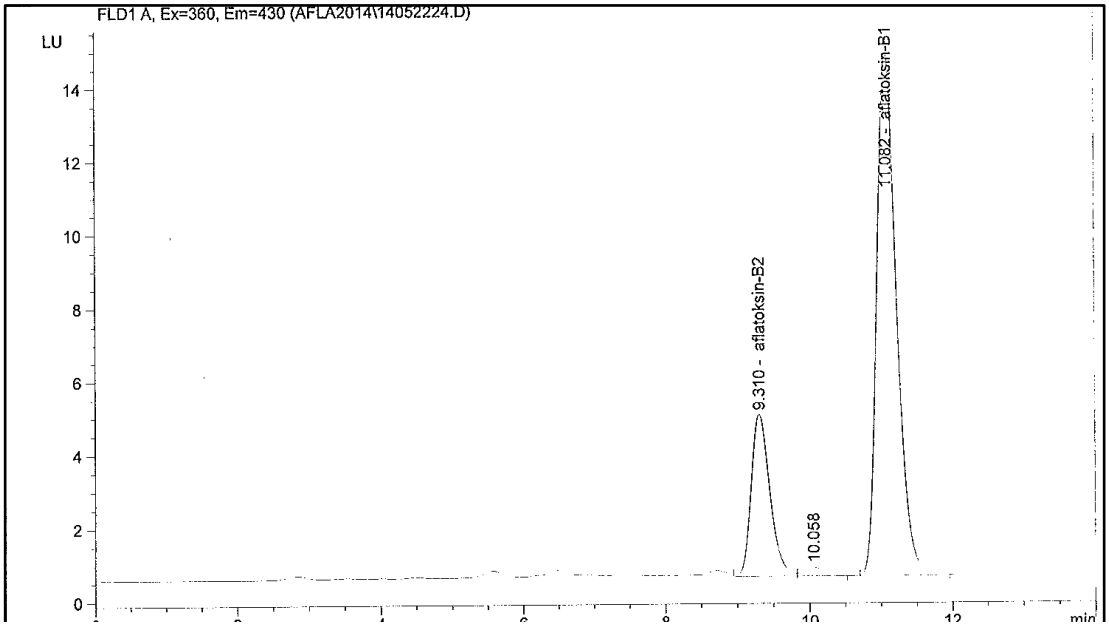

Figure A.24 Dry Roasting at 120°C for 90 minutes with the addition of 5% NaOH

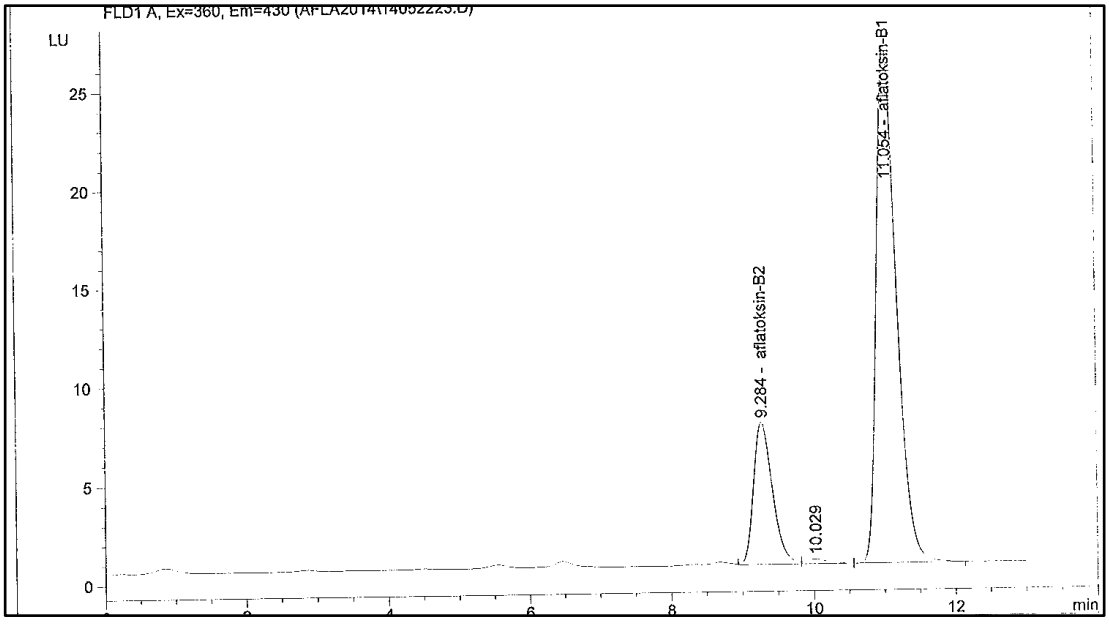

Figure A.25 Dry Roasting at 160°C for 30 minutes with the addition of 3% NaOH

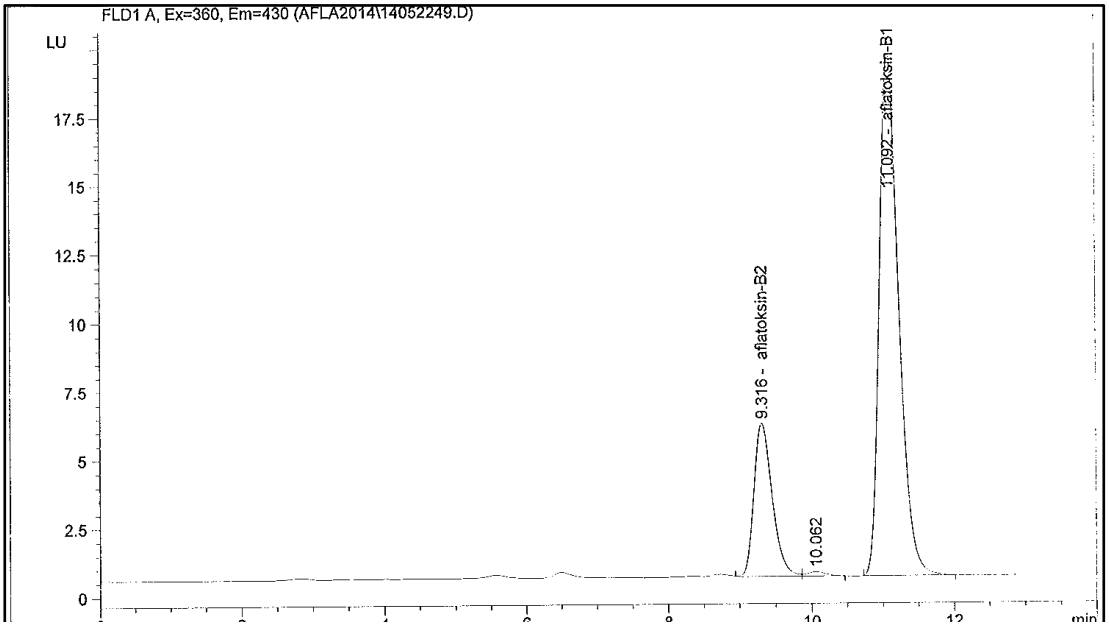

Figure A.26 Dry Roasting at 160°C for 60 minutes with the addition of 3% NaOH

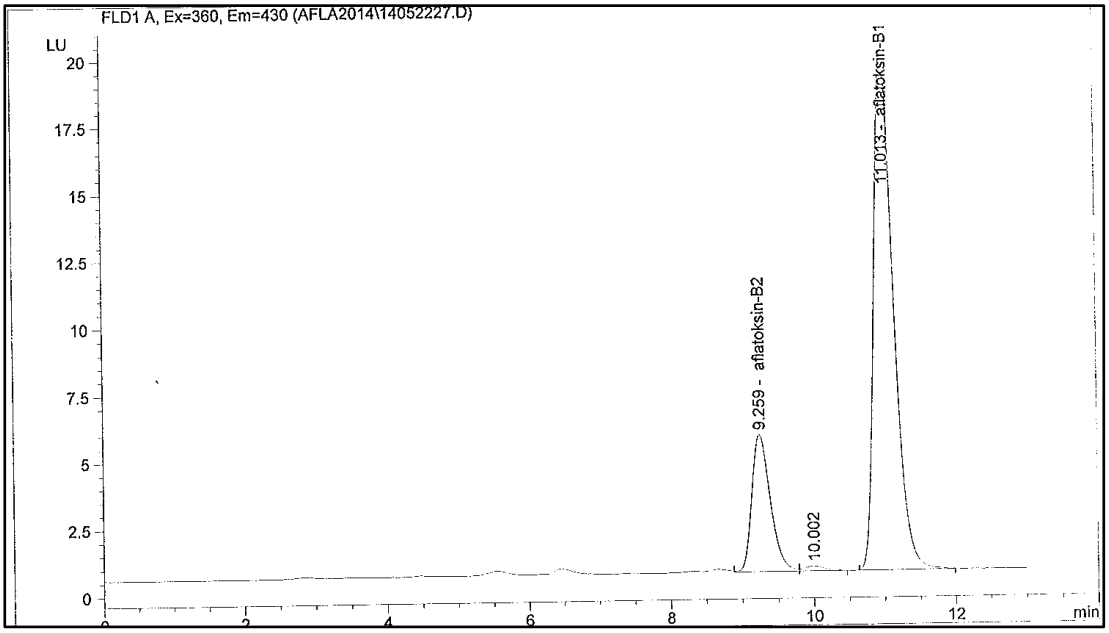

Figure A.27 Dry Roasting at 160°C for 30 minutes with the addition of 5% NaOH

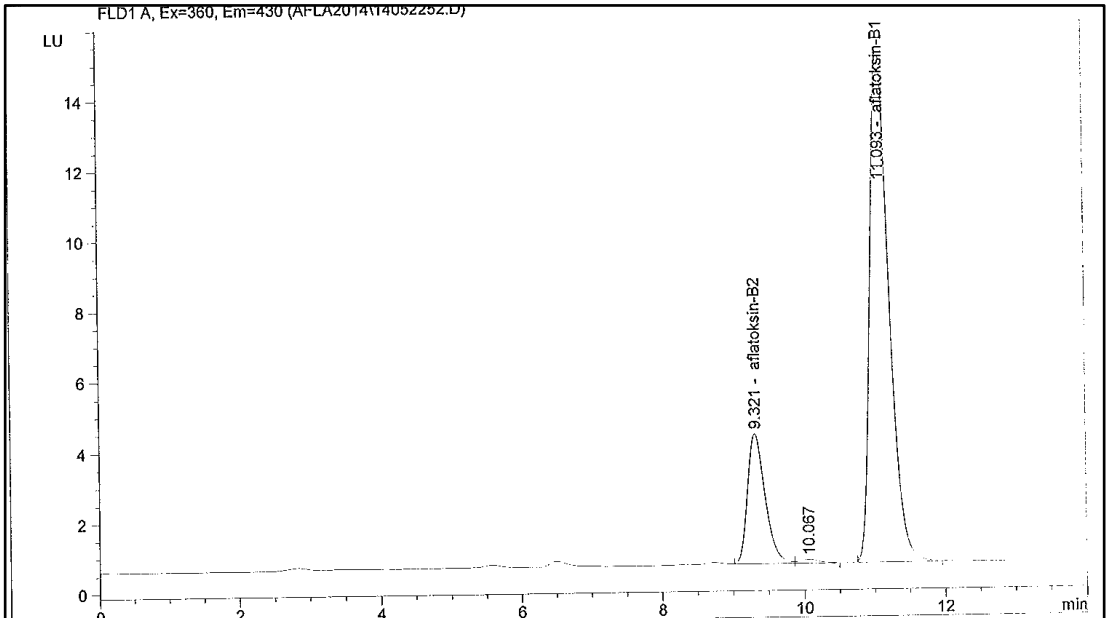

Figure A.28 Dry Roasting at 160°C for 60 minutes with the addition of 5 % NaOH

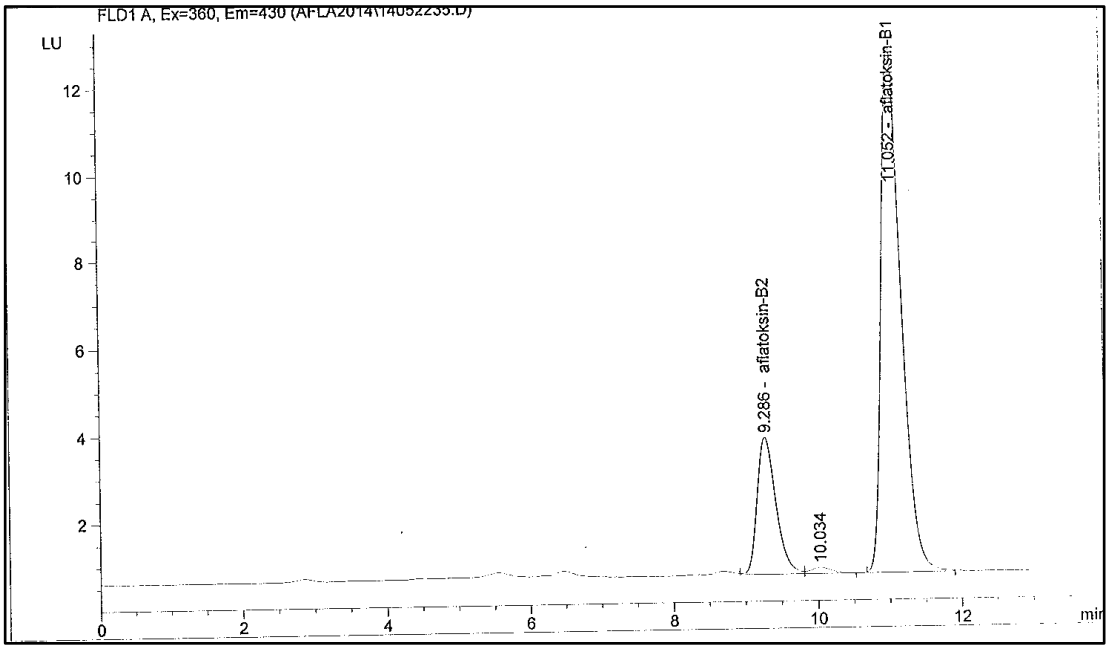

Figure A.29 Dry Roasting at 200°C for 5 minutes with the addition of 3% NaOH

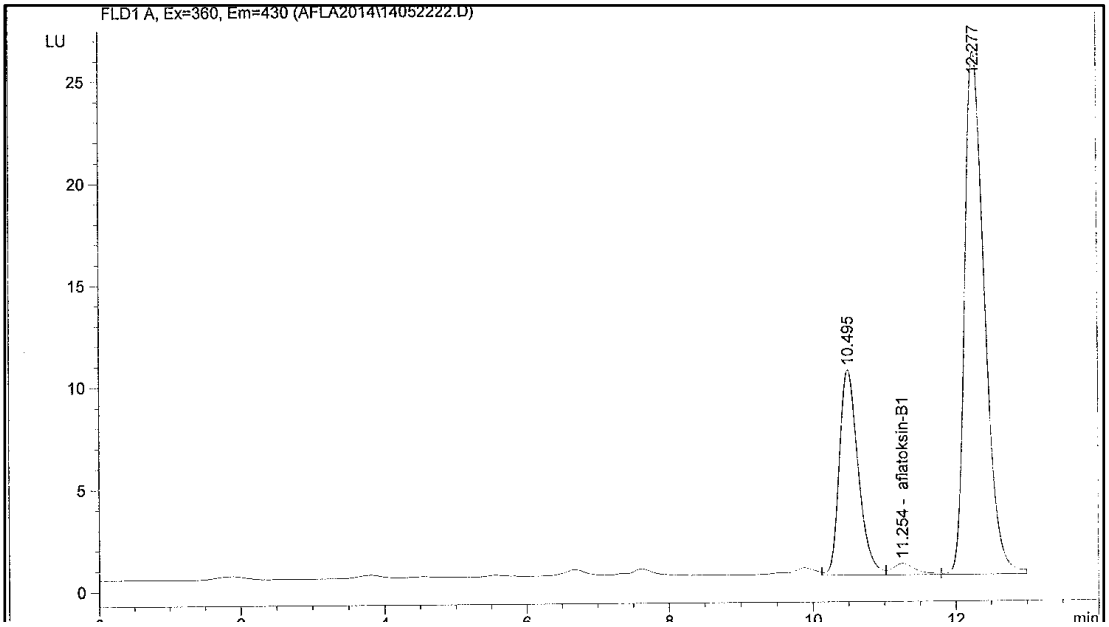

Figure A.30 Dry Roasting at 200°C for 5 minutes with the addition of 5% NaOH

APPENDIX B

x-axis represents time (minutes) and y- axis represents absorbance (nm) in graphs given in Appendix B.

HPLC GRAPHS OF CAPILLARY TUBE HEATED AFLATOXIN ANALYSES

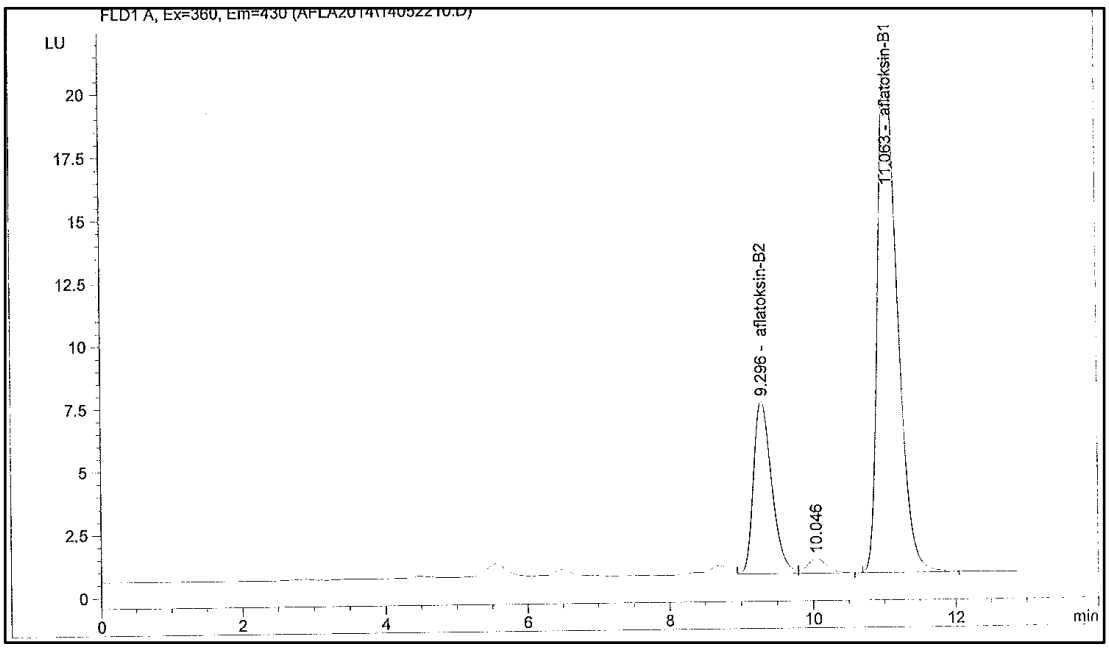

Figure B.1 Capillary Tube Heating at 120°C for 30 minutes

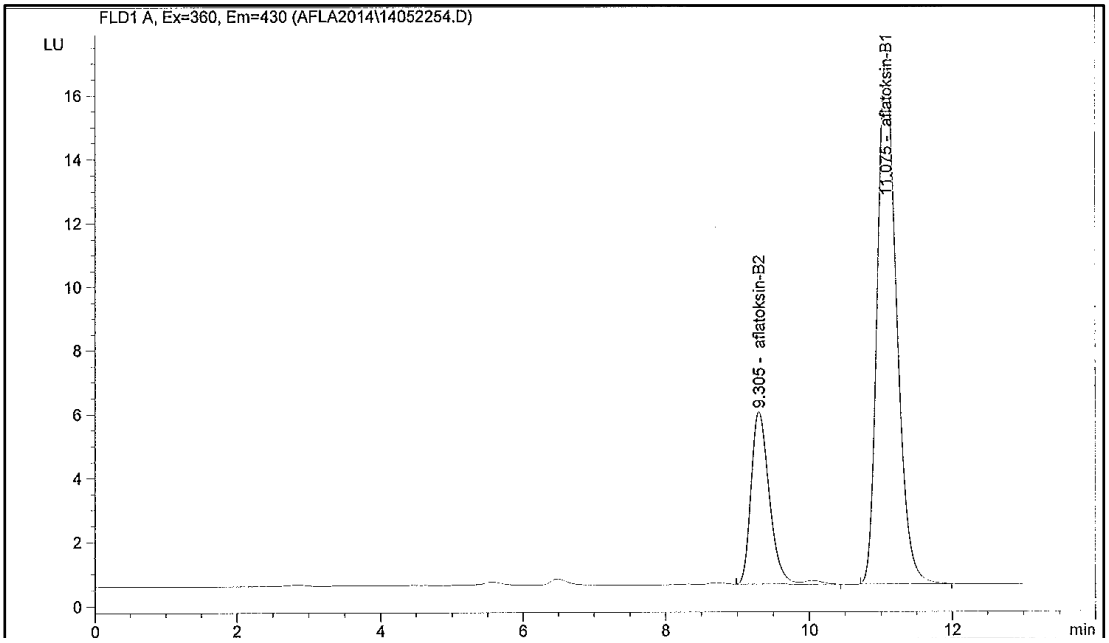

Figure B.2 Capillary Tube Heating at 120° for 60 minutes

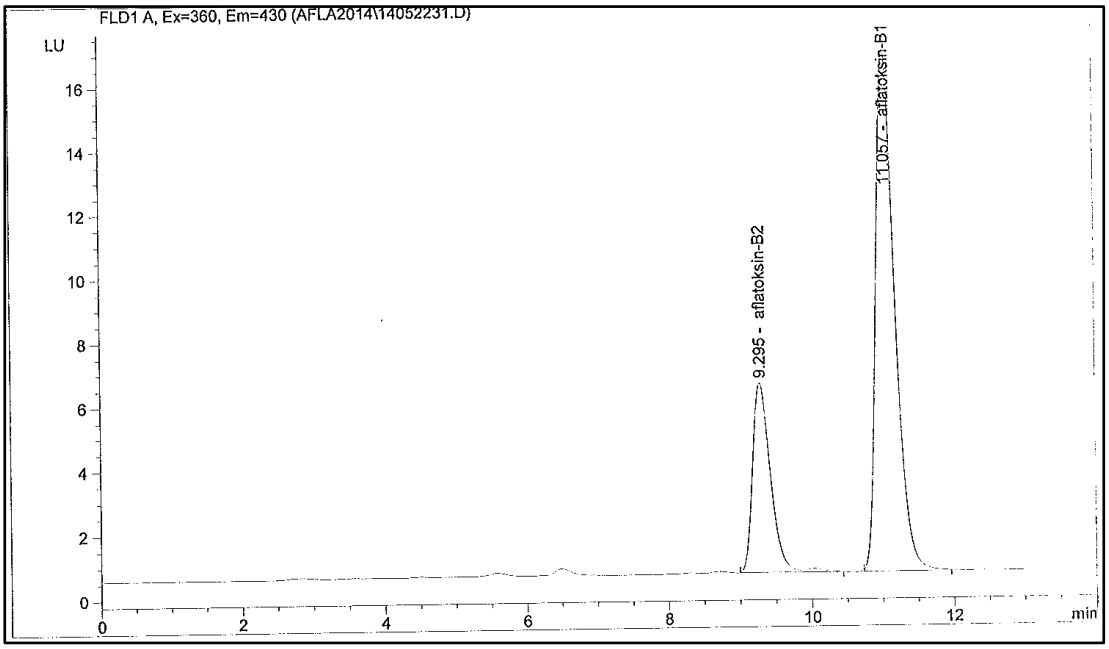

Figure B.3 Capillary Tube Heating at 120° for 90 minutes

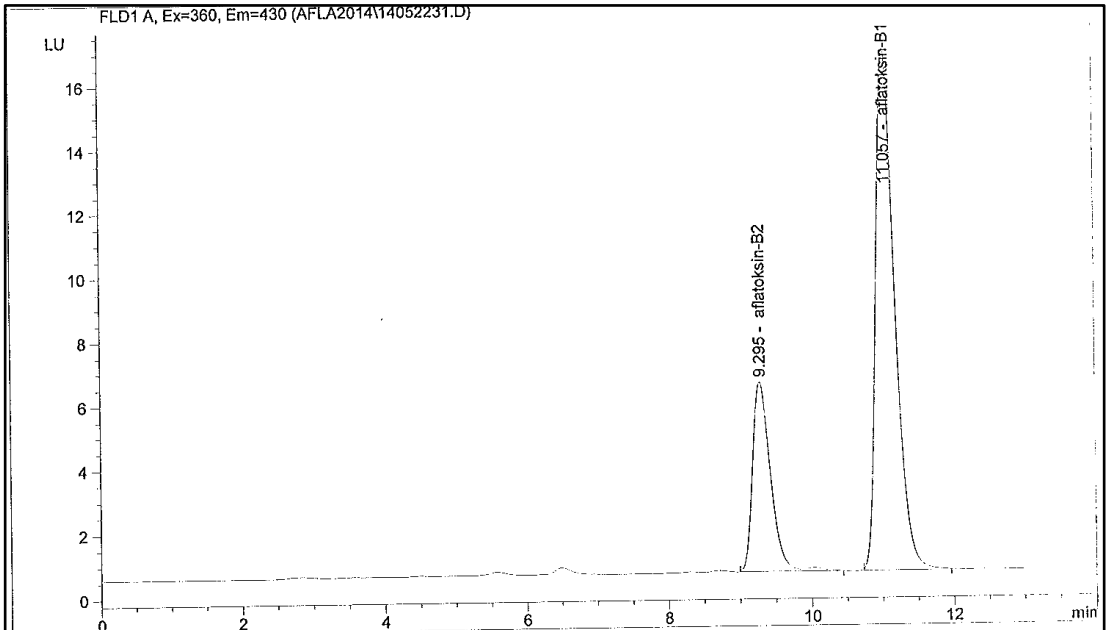

Figure B.4 Capillary Tube Heating at 160°C for 30 minutes

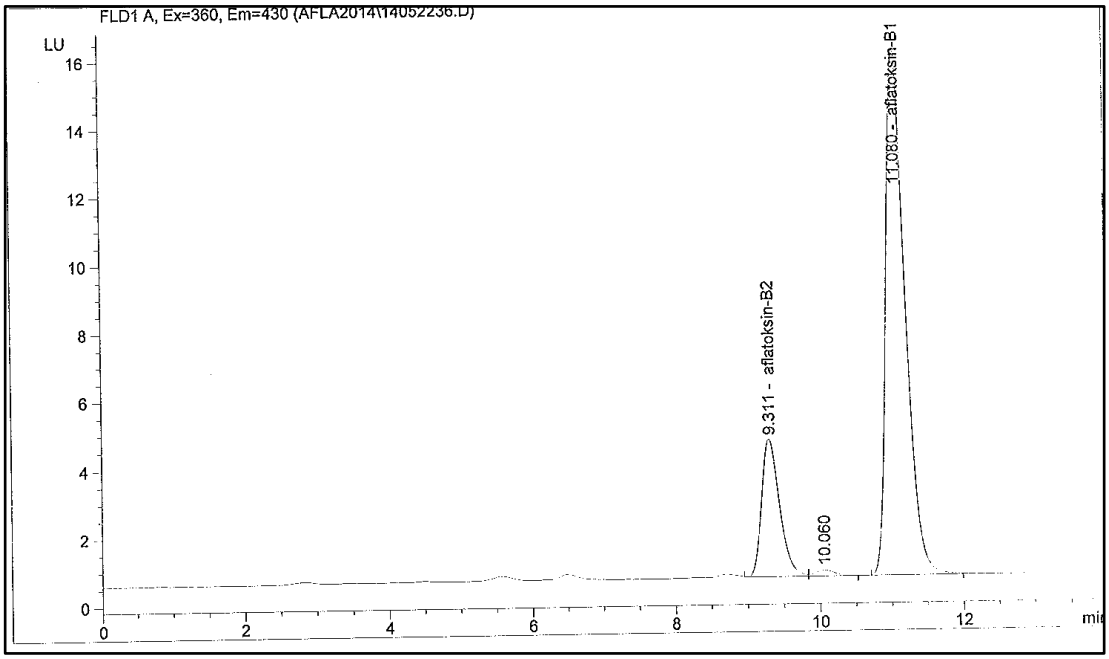

Figure B.5 Capillary Tube Heating at 160°C for 60 minutes

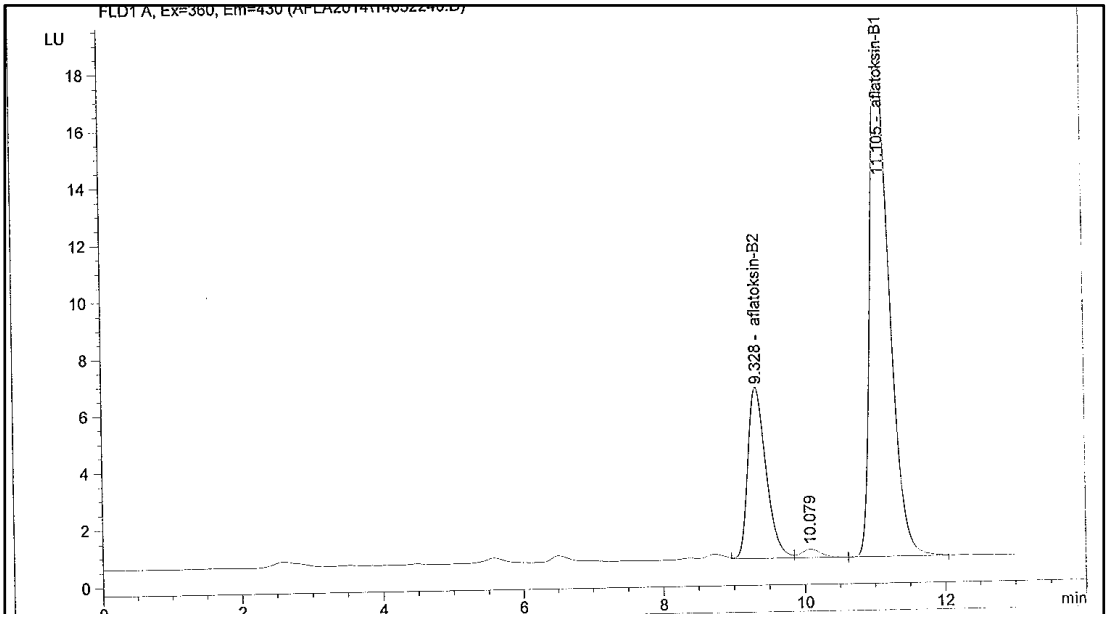

Figure B.6 Capillary Tube Heating at 200°C for 5 minutes

APPENDIX C

x-axis represents time (minutes) and y- axis represents absorbance (nm) in graph given in Appendix C.

HPLC GRAPHS OF GAMMA IRRADIATED AFLATOXIN ANALYSES

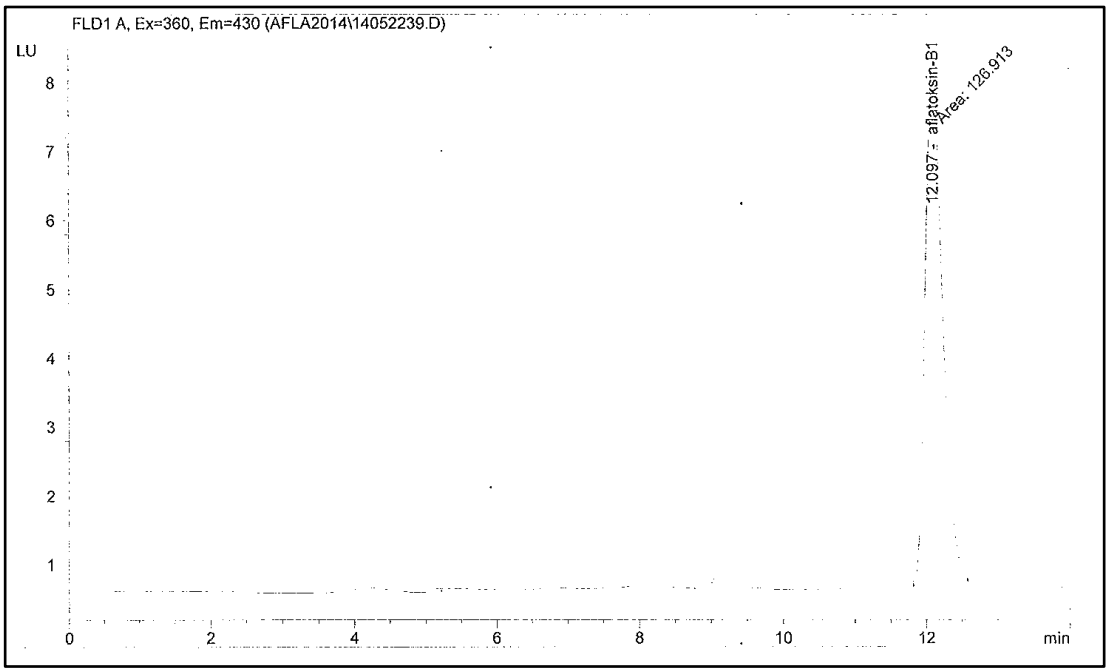

Figure C.1 Gamma Irradiation with 10 kGy

APPENDIX D

STATISTICAL ANALYSES

Chemical Type Dependent

NaOH

Number of levels: 2; 3; 3

General Linear Model: aflatoxinversustemperature; time; chemamount

Factor	Type	Levels	Values
T(°C)	fixed	2	120; 160
t(min)	fixed	3	30; 60; 90
Chemamount	fixed	3	0; 3; 5

Analysis of Varianceforaflatoxin, usingAdjusted SS forTests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
T(°C)	1	1276.9	1276.9	1276.9	8.3	0.000
t(min)	2	6464.9	6464.9	3232.5	21.01	0.000
Chemamount	2	6292.0	6292.0	3146.0	20.44	0.000
Temperature*time	2	144.8	144.8	72.4	0.47	0.632
Temperature* chemamount	2	299.4	299.4	149.7	0.97	0.397
Time*chemamount	4	523.4	523.4	130.9	0.85	0.512
Temp*time*chemamount	4	710.0	710.0	177.5	1.15	0.364
Error	18	2769.9	2769.9	153.9		
Total	35	18481.3				

S = 12.4049 R-Sq = 95.01% R-Sq(adj) = 91.86%

$Ca(OH)_2$

Source	DF	Seq SS	Adj SS	Adj MS	F	P
T(°C)	1	1027.2	1027.2	1027.2	7.00	0.016
t(min)	2	5163.8	5163.8	2581.9	17.59	0.000
Chemamount	2	8856.7	8856.7	4428.3	30.17	0.000
Temperature*time	2	66.1	66.1	33.1	0.23	0.801
Temperature* chemamount	2	174.5	174.5	87.3	0.59	0.562
Time*chemamount	4	186.6	186.6	46.7	0.32	0.862
Temp*time*chemamount	4	174.0	174.0	43.5	0.30	0.876
Error	18	2641.9	2641.9	146.8		
Total	35	18290.8				

S = 12.1149 R-Sq = 95.56% R-Sq(adj) = 91.92%

Temperature Dependent

T=120
General Linear Model: af120versus time; chemtype; chemamount

Factor	Type	Levels	Values
t(min)	fixed	3	30; 60; 90
chemtype	fixed	2	1; 2
Chemamount	fixed	3	1; 2; 3

Analysis of Variancefor af120, usingAdjusted SS forTests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
time	2	4253.8	4253.8	2126.9	11.84	0.001
Chemtype	1	145.6	145.6	145.6	0.81	0.380
Chemamount	2	4459.6	4459.6	2229.8	12.41	0.000
time*chemtype	2	62.0	62.0	31.0	0.17	0.843
time*chemamount	4	251.3	251.3	62.8	0.35	0.841
Chemtype*chemamount	2	118.7	118.7	59.4	0.33	0.723
Time*chemtype* chemamount	4	327.5	327.5	81.9	0.46	0.767
Error	18	3234.6	3234.6	179.7		
Total	35	12853.2				

S = 13.4051 R-Sq = 94.83% R-Sq(adj) = 91.07%
1:NaOH 1:0 2:3 3:5

2: Ca(OH)2 1:0 2:0.2 3:0.8

T:160

General Linear Model: af160 versus time; chemtype; chemamount

Analysis of Variancefor af160, usingAdjusted SS forTests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
time	2	6064.1	6064.1	3032.1	17.28	0.000
Chemtype	1	67.5	67.5	67.5	0.38	0.543
Chemamount	2	9671.8	9671.8	4835.9	27.56	0.000
time*chemtype	2	239.0	239.0	119.5	0.68	0.519
time*chemamount	4	512.3	512.3	128.1	0.73	0.583
Chemtype*chemamount	2	146.9	146.9	73.4	0.42	0.664
Time*chemtype* chemamount	4	276.9	276.9	69.2	0.39	0.810
Error	18	3158.1	3158.1	175.4		
Total	35	20136.6				

S = 13.2457 R-Sq = 91.32% R-Sq(adj) = 89.50%

Time Dependent

t=30

Source	DF	Seq SS	Adj SS	Adj MS	F	P
T(°C)	1	633.45	633.45	633.45	6.85	0.022
Chemtype	1	49.02	49.02	49.02	0.53	0.480
Chemamount	2	4290.72	4290.72	2145.36	23.21	0.000
T*chemtype	1	1.26	1.26	1.26	0.01	0.909
T*chemamount	2	108.12	108.12	54.06	0.58	0.572
Chemtype*chemamount	2	2028.19	2028.19	1014.09	10.97	0.002
T*chemtype* chemamount	2	511.89	511.89	255.94	2.77	0.103
Error	12	1109.22	1109.22	92.44		
Total	23	8731.88				

S = 9.61433 R-Sq = 95.30% R-Sq(adj) = 87.65%

t=60

Source	DF	Seq SS	Adj SS	Adj MS	F	P
T(°C)	1	842.5	842.5	842.5	6.09	0.030
Chemtype	1	25.2	25.2	25.2	0.18	0.677
Chemamount	2	4265.1	4265.1	2132.6	15.42	0.000
T*chemtype	1	101.7	101.7	101.7	0.74	0.408
T*chemamount	2	635.7	635.7	317.9	2.30	0.143
Chemtype*chemamount	2	88.6	88.6	44.3	0.32	0.732
T*chemtype* chemamount	2	34.7	34.7	17.4	0.13	0.883

Error	12	1659.6	1659.6	138.3
Total	23	7653.2		

S = 11.7600 R-Sq = 90.32% R-Sq(adj) = 88.44%

t=90

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
T(°C)	1	819.00	819.00	819.00	53.93	0.000
Chemtype	1	309.60	309.60	309.60	20.39	0.060
Chemamount	2	6767.11	6767.11	3383.56	222.82	0.000
T*chemtype	1	1.82	1.82	1.82	0.12	0.736
T*chemamount	2	281.62	281.62	140.81	9.27	0.004
Chemtype*chemamount	2	146.36	146.36	73.18	4.82	0.029
T*chemtype* chemamount	2	567.37	567.37	283.69	18.68	0.000
Error	12	182.22	182.22	15.19		
Total	23	9075.11				

S = 3.89679 R-Sq = 97.99% R-Sq(adj) = 96.15%

Tukey Test Result

$Ca(OH)_2$Dependent:

ChemicalAmountSignificance:

C3	**N**	**Mean**	**Grouping**
0	10	139.85	A
0.2	10	112.62	AB
0.8	10	96.79	B

Time Significance:

C2	**N**	**Mean**	**Grouping**
30	12	135.29	A
60	12	117.92	A
90	6	110.90	AB

5	6	82.2	B

TemperatureSignificance:

C1	**N**	**Mean**	**Grouping**
120	12	125.12	A
160	12	120.48	A
200	6	82.2	B

NaOHDependent:

ChemicalAmountSignificance:

C3	**N**	**Mean**	**Grouping**
0	10	141,48	A
3	10	114.66	AB
5	10	104.1	B

Time Significance:

C2	**N**	**Mean**	**Grouping**
30	12	141.43	A
60	12	118.25	A
90	6	109.90	AB
5	6	81.0	B

TemperatureSignificance:

C1	**N**	**Mean**	**Grouping**
120	12	134,82	A
160	12	124,87	A
200	6	81,0	B

APPENDIX E

EXTRACTION OF AFLATOXIN

AOAC Official Method 991.31

- Weighting of 25 gr sample
- Milling
- Addition of 125 ml methanol/ water solution (70/30)
- Shaking 2 minutes
- Filter with Whatman 4 paper
- Taking 15 ml of filtered sample and add 30 ml distilled water
- Taking 15 ml of prepared solution and add 10 ml distilled water

Figure E1. Extraction of Aflatoxin

- Separation of aflatoxin by immunoaffinity colons (AFLAPREP immunoaffinity columns were used for detection) (Figure E2)
    - *The columns contain a gel suspension of monoclonal antibody specific to the toxins of interest. Following extraction of the toxins the sample extract is filtered, diluted and passed slowly through the*

immunoaffinity column. Any toxins which are present in the sample are retained by the antibody within the gel suspension. The column is washed to remove unbound material and the toxins are then released from the column following elution with solvent. The eluate is collected prior to analysis by HPLC or LC-MS/MS. Aflatoxins are required to be derivatised when analysed by HPLC (r-biopharm).

Recovery by;

- Addition of 1 ml methanol
- Addition of 1 ml of distilled water

Figure E2. Separation of Aflatoxin by Immunoaffinity Colons

HPLC Conditions:

Column:	Zorbax Eclipse Plus C18, 4.6 x 150 mm x 5 µm
Mobile Phase A:	1L water containing 238 mg KBr and 700 µL 4M HNO_3
Mobile Phase B:	MeOH
Isocratic:	A：B = 50 : 50
Flow rate:	1.0 mL/min
Detection:	Ex: 360 nm, Em: 430 nm
Injection:	100 µL
Reaction coil:	0.5 mm i.d.*34 cm long peek tubing(from the exit of KOBRA cell to the entrance of FLD)

APPENDIX F

Table F. 1Data Obtained from Roasting at 120/ 160/ 200°C for Different Time Intervals with the Addition of 3 and 5% NaOH

Temperature	NaOH Amount (%)	Time (min)	B1 Amount (ppb)	Temperature	NaOH Amount (%)	Time (min)	B1 Amount (ppb)	Temperature	NaOH Amount (%)	Time (min)	B1 Amount (ppb)
120°C	0	30	165±7.07	160°C	0	30	148.8±14.14	200°C	0	5	112.4±7.64
	0	60	134.2±6.79		0	60	136.8±10.46		3	5	75.4±12.63
	0	90	131.7±0.07		3	30	130±7.78		5	5	45±7.72
	3	30	135.5±0.99		3	60	105.8±3.11				
	3	60	126.6±1.42		5	30	131.4±0.92				
	3	90	124.4±2.12		5	60	91.9±9.34				
	5	30	133.4±2.32								
	5	60	114.2±0.79								
	5	90	96.8±0.42								

Table F. 2Data Obtained from Roasting at 120/ 160/ 200°C for Different Time Intervals with the Addition of 0.2 and 0.8% $Ca(OH)_2$

Temperature	$Ca(OH)_2$ Amount	Time (min)	B1 Amount (ppb)	Temperature	$Ca(OH)_2$ Amount	Time (min)	B1 Amount (ppb)	Temperature	$Ca(OH)_2$ Amount	Time (min)	B1 Amount (ppb)
120°C	0	30	165±7.37	160°C	0	30	148.8±1.14	200°C	0	5	112.4±10.46
	0	60	134.2±0.79		0	60	136.8±1.46		0.2	5	81±23.47
	0	90	131.7±0.07		0.2	30	126.5±7.07		0.8	5	43±8.49
	0.2	30	137.3±6.36		0.2	60	107.6±7.64				
	0.2	60	122.4±0.84		0.8	30	109.35±1.95				
	0.2	90	100.9±9.05		0.8	60	93.8±2.31				
	0.8	30	124.8±9.89								
	0.8	60	112.7±2.94								
	0.8	90	97.1±0.28								

Table F. 3 Data Obtained from Capillary Tube Heating at 120/ 160/ 200°C for Different Time Intervals

Temperature	Time (min)	B1 Amount (ppb)	Temperature	Time (min)	B1 Amount (ppb)	Temperature	Time (min)	B1 Amount (ppb)
120°C	30	144.6	160°C	30	143.5	200°C	5	120.5
	60	115.5		60	112.2			
	90	101.8						

CIRRICULUM VITAE

PERSONAL INFORMATION

Surname/ Name:	AYGÜN/ Şükran Gizem
Nationality:	TC
Date and Place of Birth:	9 March 1985, Ankara
Phone:	05062166065
email:	e139629@metu.edu.tr/ aygngizem@gmail.com

EDUCATION

Degree	Institution	Year of Graduation
MSc	METU Food Engineering	2010
BS	METU Food Engineering	2008
Minor Program	METU Department of Biology	2007

WORK EXPERIENCE

Year	Place	Enrollment
2012- Present	Ministry of Food, Agriculture and Livestock	Food Engineer
2009-2012	METU Food Engineering Department	Research Assistant
2008	Bağdat Baharat	Expert of Import
2007- August	TUBİTAK	Intern
2006- July	Yayla Pasta Factory	Intern
2006- February	Ali Uzun Chocolate and Confectionary Factory	Intern

FOREIGN LANGUAGES

Advanced English, basic Spanish, basic German

CONGRESS

2nd Food Security Congress (Oral Presentation)- İstanbul/ 2010
Detection of Gamma Irradiation in Spiceswith OSL MethodanditsReliability

7th FoodEngineeringCongress (Oral Presentation)- Ankara/ 2011
Detection of Gamma Irradiation in Spiceswith OSL MethodanditsReliability

Project of ScienceHeroes (Oral Presentation)- Ankara/ 2011
Food Security from Farm toFork

3rd Food Security (Poster Presentation)- İstanbul/ 2012

Development of Continuous UV SystemandTestingtheeffects of UV Radiation on SpiceMicroflora