**GAZİANTEP UNIVERSITY GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES**

# EFFECT OF HIGH HYDROSTATIC PRESSURE ON AFLATOXIN CONTENT IN DRIED RED PEPPER

**M. Sc. THESIS**
**IN**
**FOOD ENGINEERING**

BY
**NENE MELTEM KEKLİK**
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# Effect of High Hydrostatic Pressure on Aflatoxin Content in Dried Red Pepper

**M.Sc. Thesis**

**in**

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**Supervisor**

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## ABSTRACT

### EFFECT OF HIGH HYDROSTATIC PRESSURE ON AFLATOXIN CONTENT IN DRIED RED PEPPER

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M.Sc. in Food Engineering

Supervisor: Prof. Dr. Şükrü KARATAŞ

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In this study, the effect of high hydrostatic pressure on total aflatoxin and $B_1$, $B_2$, $G_1$, and $G_2$ types in dried red pepper was investigated with three varying parameters: pressure (150-350 MPa), time (4-12 min), and temperature (20, 30, and 40 ±2 ºC).

In the experiments, it was observed that the percentage of total aflatoxin and $B_1$ decreased almost in the same manner as pressure, time, and temperature increased. However, this pattern was not observed for concentrations of $B_2$, $G_1$, and $G_2$. It was found that the most effective pressure, time, and temperature for the decrease in total aflatoxin and $B_1$ were 350 MPa, 12 minutes, and 40 ±2 ºC, which caused 49 % decrease in both total aflatoxin and $B_1$ concentrations.

A zero-order expression described the decrease rate of total aflatoxin during each pressuring period. Arrhenius expressions described the effect of temperature on each pressurizing period and rate constants of total aflatoxin decrease. It was confirmed by the experimental results that the reaction rate increased as the pressure and temperature were increased, whereas the activation energy showed decrease as the pressure was increased.



**Keywords**: Aflatoxin, high hydrostatic pressure, dried red pepper

## ÖZET

## YÜKSEK HİDROSTATİK BASINCIN KURUTULMUŞ KIRMIZI BİBERDE BULUNAN AFLATOKSİN MİKTARI ÜZERİNE ETKİSİ

KEKLİK, Nene Meltem

Yüksek Lisans Tezi, Gıda Mühendisliği

Tez Yöneticisi: Prof. Dr. Şükrü KARATAŞ

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Bu çalışmada, yüksek hidrostatik basıncın kurutulmuş kırmızı biber içerisindeki toplam aflatoksin ve $B_1$, $B_2$, $G_1$, ve $G_2$ türleri üzerine etkisi üç değişken parametre ile araştırılmıştır: Bu parametreler, basınç (150-350 MPa), zaman (4-12 dk), ve sıcaklıktır (20, 30, ve 40 ±2 ºC).

Yapılan deneylerde; basınç, zaman, ve sıcaklık arttığında, toplam aflatoksin ve $B_1$ yüzdeleri birbirine benzer şekilde düşmüştür. Ancak, bu durum $B_2$, $G_1$, and $G_2$ konsantrasyonları için gözlenmemiştir. Toplam aflatoksin ve $B_1$ türünün azalmasında en etkili basınç, zaman ve sıcaklık değerleri, hem toplam aflatoksinde, hem de $B_1$ türünde % 49 azalmaya neden olan 350 MPa, 12 dakika, ve 40 ±2 ºC olarak bulunmuştur.

Her basınçlama süresinde toplam aflatoksinin düşme hızları sıfırıncı derece denklemle tanımlanmıştır. Sıcaklığın her basınçlama süresi ve toplam aflatoksin miktarının azalma hızı üzerine etkisi Arrhenius denklemiyle tanımlanmıştır. Deney sonuçlarına göre reaksiyon hızı basınç ve sıcaklık arttıkça artmıştır. Diğer yandan, aktivasyon enerjisi ise basınç arttıkça azalma göstermiştir.



**Anahtar Kelimeler**: Aflatoksin, yüksek hidrostatik basınç, kurutulmuş kırmızı biber

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## LIST OF SYMBOLS / ABBREVIATIONS

| | |
|---|---|
| $[A]_0$ | : Initial concentration of reactant A |
| [A] | : Concentration of reactant A (total aflatoxin) |
| A | : Preexponential factor in Arrhenius expression |
| $E_a$ | : Activation energy (kcal/gmol) |
| k | : Reaction rate constant |
| R | : Gas constant (1.987 cal/gmol.K) |
| T | : Temperature (Kelvin, K) |
| t | : Time (min) |
| C18 | : 18 Carbon atoms in the chain |
| ca. | : Approximately |
| IU | : International Units |
| $LD_{50}$ | : Lethal Dose to kill 50 % of the total population |
| afla. | : Aflatoxin |
| mic | : Micron (micrometer) |
| ppb | : Parts per billion (μg/kg) |
| Temp. | : Temperature |
| vs. | : Versus |
| wb | : Wet basis |

# CHAPTER 1

# THEORETICAL BACKGROUND

## 1.1. Aflatoxin

Aflatoxin is a naturally occurring mycotoxin produced by two types of molds: Aspergillus flavus (Appendix Figure B.1) and Aspergillus parasiticus (Appendix Figure B.2). Aspergillus flavus is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature (U.S. Department of Agriculture, 2006). Presently, 18 different types of aflatoxin have been identified in nature with aflatoxin $B_1$ considered as the most toxic (Guerzoni, 1999). While the presence of Aspergillus flavus does not always indicate harmful levels of aflatoxin it does mean that the potential for aflatoxin production is present (U.S. Department of Agriculture, 2006).

### 1.1.1. Physical and chemical properties of aflatoxins

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites mainly by the fungus Aspergillus flavus and Aspergillus parasiticus on a variety of food products. Among 18 different types of aflatoxin identified, major members are aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$. Aflatoxin $B_1$ ($AFB_1$) is normally predominant in amount in cultures as well as in food products. Pure $AFB_1$ is pale-white to yellow crystalline, odorless solid.

Aflatoxins are soluble in methanol, chloroform, acetone and acetonitrile. Aspergillus flavus typically produces $AFB_1$ and Aflatoxin $B_2$ ($AFB_2$), whereas Aspergillus parasiticus produce Aflatoxin $G_1$ ($AFG_1$) and Aflatoxin $G_2$ ($AFG_2$) as well as $AFB_1$ and $AFB_2$. Four other aflatoxins $M_1$, $M_2$, $B_2A$, $G_2A$ which may

be produced in minor amounts were subsequently isolated from culture of Aspergillus flavus and Aspergillus parasiticus.

A number of closely related compounds namely aflatoxin $GM_1$, parasiticol and aflatoxicol are also produced by Aspergillus flavus. Aflatoxin $M_1$ and $M_2$ are major metabolites of aflatoxin $B_1$ and $B_2$ respectively, found in milk of animals that have consumed feed contaminated with aflatoxins.

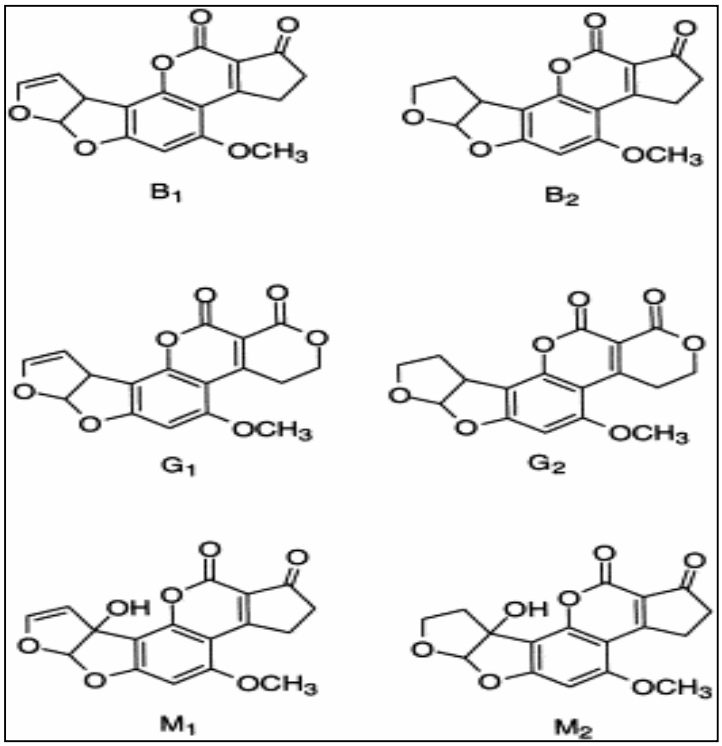


Figure 1.1 Chemical structures of the most frequently occurring forms of aflatoxin (Reddy and Farid, 2000)

Aflatoxins are normally referred to the group of difuranocoumarins and classified in two broad groups according to their chemical structure; the difurocoumarocyclopentenone series ($AFB_1$, $AFB_2$, $AFB_2A$, $AFM_1$, $AFM_2$, $AFM_2A$ and Aflatoxicol) and the difurocoumarolactone series ($AFG_1$, $AFG_2$, $AFG_2A$, $AFGM_1$, $AFGM_2$, $AFGM_2A$ and $AFB_3$). Aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of;

$AFB_1 > AFG_1 > AFB_2 > AFG_2$

as illustrated by their $LD_{50}$ (18.2, 84.8, 392 and 172.5 mg of $B_1$, $G_1$, $G_2$ and $B_2$, respectively) values for ducklings. Structurally the dihydrofuran moiety, containing double bond, and the constituents like the coumarin moiety are of importance in producing biological effects (Figure 1.1). Aflatoxins fluoresce strongly in ultraviolet light (approximately (ca.) 365 nm); $B_1$ and $B_2$ produce a blue fluorescence where as $G_1$ and $G_2$ produce green fluorescence (Reddy and Farid, 2000).

Table 1.1 Chemical and physical properties of aflatoxins (Reddy and Farid, 2000)

| **Aflatoxin** | **Molecular formula** | **Molecular weight** | **Melting point** |
|---|---|---|---|
| $B_1$ | $C_{17}H_{12}O_6$ | 312 | 268-269 |
| $B_2$ | $C_{17}H_{14}O_6$ | 314 | 286-289 |
| $G_1$ | $C_{17}H_{12}O_7$ | 328 | 244-246 |
| $G_2$ | $C_{17}H_{14}O_7$ | 330 | 237-240 |
| $M_1$ | $C_{17}H_{12}O_7$ | 328 | 299 |
| $M_2$ | $C_{17}H_{14}O_7$ | 330 | 293 |
| $B_2A$ | $C_{17}H_{14}O_7$ | 330 | 240 |
| $G_2A$ | $C_{17}H_{14}O_8$ | 346 | 190 |

### 1.1.2. Reactions of aflatoxins

Aflatoxins in dry state are very stable to heat up to their average melting point (ca. 250 ˚C) (Table 1.1). However, in the presence of moisture and at elevated temperatures there is destruction of aflatoxin over a period of time. Such destruction can occur either with aflatoxin in oilseed meals, aflatoxin in roasted peanuts or aflatoxin in aqueous solution at pH 7. Although the reaction products have not been examined in detail, it seems likely that such treatment leads to opening of the lactone ring with the possibility of decarboxylation at elevated temperatures. The metabolites include aflatoxin $D_1$ and $CO_2$ (Cucullu et al., 1976).

In alkali solution hydrolysis of the lactone moiety occurs. This hydrolysis appears to be reversible, since it has been shown that recyclization occurs following

acidification of a basic solution containing aflatoxin. At higher temperatures (ca. 100 ˚C) ring opening followed by decarboxylation occurs and reaction may proceed further, leading to the loss of the methoxy group from the aromatic ring. Similar series of reactions also seems to occur with ammonia and various amines.

In the presence of mineral acids, aflatoxin $B_1$ and $G_1$ are converted into aflatoxin $B_2A$ and $G_2A$ due to acid-catalyzed addition of water across the double bond in the furan ring. In the presence of acetic anhydride and hydrochloric acid; the reaction proceeds further to give the acetoxy derivative. Similar adducts of aflatoxin $B_1$ and $G_1$ are formed with formic acid-thionyl chloride, acetic acid-thionyl chloride and trifluoroacetic acid.

Many oxidizing agents, such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perborate react with aflatoxin and change the aflatoxin molecule in some way as indicated by the loss of fluorescence. The mechanisms of these reactions are uncertain and the reaction products remain unidentified in most cases.

Hydrogenation of aflatoxin $B_1$ and $G_1$ yields aflatoxin $B_2$ and $G_2$, respectively. Further reduction of aflatoxin $B_1$ by 3 moles of hydrogen yields tetrahydroxyaflatoxin. Reduction of aflatoxin $B_1$ and $B_2$ with sodium borohydride yielded aflatoxin $RB_1$ and $RB_2$, respectively. These arise as a result of opening of the lactone ring followed by reduction of the acid group and reduction of the keto group in the cyclopentene ring (Reddy and Farid, 2000).

### 1.1.3. Measurement techniques and HPLC for aflatoxin analysis

There are currently several different accepted measurement techniques available for determining aflatoxin levels. In general, most measurement techniques require three steps: extraction to remove the aflatoxin from complex mixtures of materials in which it is found; purification to remove interferents and finally detection and quantification.

The more traditional techniques used to purify aflatoxin before presenting it to a detector for quantification utilize some form of chromatography, a mainstay of

analytical chemistry. Examples include, thin layer chromatography (TLC), gas chromatography (GC), and high pressure liquid chromatography (HPLC). These methods are well-proven and widely accepted; however, they are often viewed as laborious and time intensive, requiring a significant investment in equipment, materials, and maintenance.

Some other modern methods of sample extraction and purification rely on immunochemical techniques. These techniques utilize aflatoxin-specific antibodies to efficiently extract and purify aflatoxin from complex mixtures. Antibodies are fundamental components of immune systems which protect against various disease causing organisms and foreign molecules. They are proteinaceous biomolecules produced by a host animal's immune system that identify and tightly bind a specific antigen or target molecule, such as a coat protein on a flu-causing virus. Once bound, the antibody–antigen complex is marked for destruction by other elements of the immune system (Bargeron et al., 1999). Outside the body, this same antibody specificity has been exploited by microbiologists to enable faster sample preparation and provide for very sensitive antigen identification schemes. Whereas the target antigen is typically some foreign protein on the surface of a bacterium, or virus, it is also relatively easy to grow antibodies that are specific for many other chemical compounds, including aflatoxin.

There are a number of immunochemical based assays that are used for detecting aflatoxin (Chu and Ueno, 1977), (Eaton and Groopman, 1994): radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and immunoaffinity column assay (ICA).

Aflatoxin analysis using HPLC (Figure 1.2 and Figure B.6) for separation and detection is quite similar to TLC because similar sampling and extraction procedures are used. The major advantages of HPLC over TLC are speed, automation, improved accuracy, and precision. Both normal-phase and reverse-phase HPLC separations have been developed for aflatoxin analyses. Early experimental works carried out by Seitz (1975) and Garner (1975) on HPLC separations revealed that aflatoxins could be separated on nominal-phase columns and detected with either a UV (ultraviolet) detector or a fluorescence detector. It is noted that the fluorescence detector had

limited usefulness for aflatoxin $B_1$ and $B_2$ with normal phase separations (Seitz, 1975).

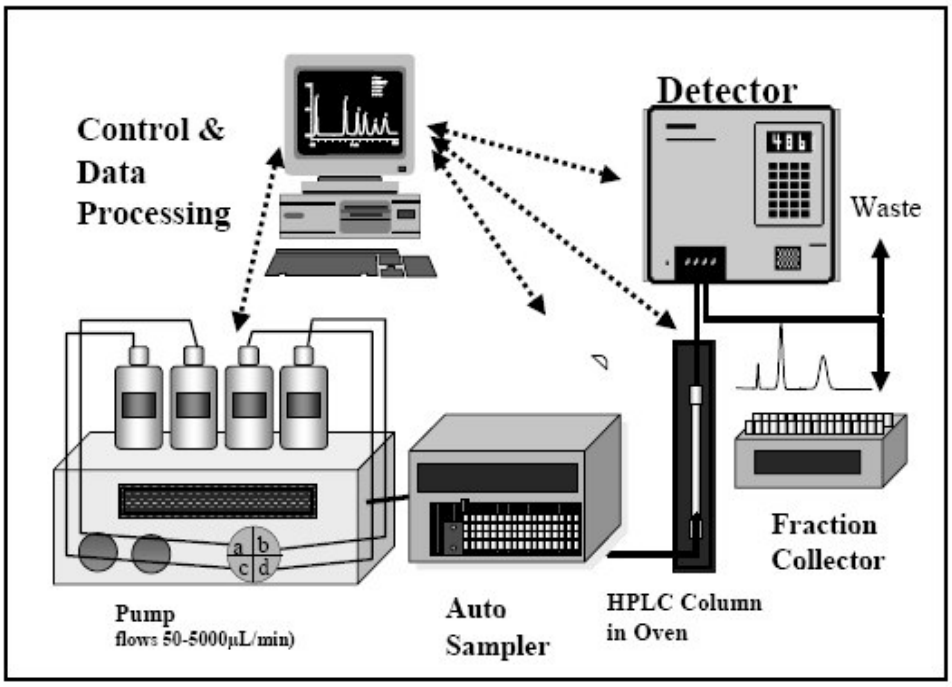


Figure 1.2 HPLC Set-up (Levin, 2006)

Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase HPLC separations. However, the fluorescence intensities of $B_1$ and $G_1$ are diminished in reverse-phase solvent mixtures so the derivatives B and G are generally prepared before injection. Analysts should be aware that derivatives B and G are not stable in methanol, which should be used with caution, especially in the injection solvent. Acetonitrile-water mixtures do not degrade B and G rapidly and are preferred to methanol-water mobile phases.

Several reverse-phase methods have been published by Cohen and Lapointe (1981) and Stubblefield and Shotwell (1977) including three comparisons to the TLC method (Trater et al., 1984). Stubblefield and Shotwell (1977) found that $M_1$, and $M_2$ as well as $B_1$, $B_2$, $G_1$, and $G_2$ could be resolved and detected with a UV detector at 350 nm using reverse-phase chromatography. The methods developed by Hutchins and Hagler (1983) and Trater et al., (1984) all use TFA (fluorescent trifluoroacetic

acid) derivatization and apparently compare favorably with other methods. Diebold et al. (1979) used laser fluorometry to detect aflatoxin B after reverse-phase chromatography. Davis and Diener (1979) found that the iodine derivative of $B_1$ could be used for confirmation and developed a reverse-phase method with fluorescence detection for this derivative. This work led to the development of a postcolumn iodination method to enhance B and G fluorescence after reverse phase chromatography. Shepard and Gilbert (1984) investigated the conditions needed for the post column iodination reaction to enhance fluorescence of aflatoxins $B_1$ and $G_1$.

### 1.1.4. Factors responsible for aflatoxin production

- The nature of the strain of the fungus, substrate, pH, temperature, relative humidity, moisture content of the substrate and aeration have been found to influence the quality and quantity of aflatoxins produced.
- The optimum limits for growth of Aspergillus flavus and Aspergillus parasiticus are 82-85 % relative humidity and temperature of 30-32 °C.
- The optimum conditions for aflatoxin production are between 25 °C and 30 °C at 85 % relative humidity.
- Fungal growth is optimum when moisture levels of the substrate range from 10 to 30 % (ikisan.com, 2000).

### 1.1.5. Hazards of aflatoxin to human health

By the early 1960s, it became evident that aflatoxins represent a significant public health concern. Although contamination from ubiquitous fungi such as Aspergillus, cannot be eliminated, exposure to the toxins can be minimized. Consequently, aflatoxin levels in animal feed and various human food products are now monitored and tightly regulated by various US government agencies. Action levels above which products are removed from commerce are typically 20 parts per billion (ppb) for human foodstuffs, with the exception of milk, which has a more stringent action level of 0.5 ppb for one of the aflatoxin metabolites ($M_1$). Somewhat higher action levels (100–200 ppb) are allowed for some livestock feeds, depending on the species' sensitivity to aflatoxins and the ultimate use of the animals (Bargeron et al., 1999).

There had been a growing body of circumstantial evidence that aflatoxin $B_1$ is carcinogenic, as well as acutely toxic, to humans, but in 1987, The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) accepted that aflatoxin should be classified as a Group 1 carcinogen. Regulatory levels set by the governments of many countries are based on the premise that aflatoxin is indeed carcinogenic and the European Community agreed on 16 July 1998 a limit of 2 $\mu g\ kg^{-1}$ for aflatoxin $B_1$ in a range of foods for human consumption.

According to a medical survey; aflatoxin $B_1$ induces the transversion of G → T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. The mutation almost exclusively consists of a G → T transversion in the third position of this codon, resulting in the insertion of serine at position 249 in the mutant protein (Aguilar, 1993).

### 1.1.6. Prevention of aflatoxin

Preventive measures constitute the best approach to minimize the contamination of food products by Aspergillus group of fungi and their toxins. The following are some of the preventive measures for avoiding aflatoxin contamination in groundnut:

- Avoiding mechanical and biological damage to the crop particularly during cultivation, harvesting and subsequent processing.
- Using supplemental irrigation during drought to avoid plant stress.
- Harvesting the crop at peak maturity and use inverted windrows to optimize curing.
- Drying the produce as rapidly as possible but the rate of drying must be controlled to prevent excessive skin slippage and splitting of kernels. When the crop is harvested under wet conditions the pods should be picked and sun-dried immediately.
- Drying food products to a safe moisture level (8 %) before storage.
- During drying, grinding and extraction of oil, batches of produce originating from different places are mixed. It is desirable to remove the contaminated pods and kernels either manually, or mechanically, or by using electronic devices. Wherever groundnut kernels or flour form a large part of the human diet as a major source of protein, the risk is reduced by hand sorting.

- Storing the product at low temperature and humidity.
- Avoiding rewetting of product by storing under dry condition and proper packing during transit.
- Based on the results obtained at the National Research Centre for Groundnut the use of rock salts (2 %), plant products like asafoetida (hing), 0.1 % if pure and 2 % if impure and turmeric powder (2 %) has been suggested for preventing aflatoxin contamination in groundnut (ikisan.com, 2000).

### 1.1.7. Detoxification of aflatoxin

Techniques that remove or destroy aflatoxins from samples must be applied only if preventive measures have failed, and not as an alternative to good agricultural and storage practices. A detoxification process must be technically and economically viable and may be used if the process:

- Does not produce or leave toxic or carcinogenic residues in the final product.
- Destroys fungal spores and mycelia which could, under favorable conditions, proliferate and form new toxins.
- Preserves the nutritive value and acceptability of the product.
- Does not significantly alter important technological properties.

Today, treatment with ammonia is the most promising process for detoxification of aflatoxin. High percentage reductions in aflatoxin levels can be obtained with this method:

- The oilseed cake containing 12-15 % moisture is treated with ammonia gas under pressures of 2 to 3 atmospheres for 15 to 30 minutes at a temperature of around 90 ˚C.
- By this method aflatoxins to the extent of 95 % get destroyed. Toxicological studies carried out on the ammonia-treated oil cakes revealed that the product realized by ammonification is safe for edible purposes (ikisan.com, 2000).

### 1.1.8. Physical techniques for aflatoxin reduction

- Physical techniques like autoclaving and exposure to gamma-irradiation were useful in reducing aflatoxins.
- Autoclaving at 120 ˚C and at 0.0105 kg/mm$^2$ is known to reduce toxins from 9,000 ppb to 350 ppb.

- Gamma-irradiation at a level of 5 M rad would destroy 80 % of the aflatoxins in a sample.
- At the CFTRI (Central Food Technological Research Institute), Mysore, a simple method for inactivation of aflatoxin in oil has been developed. In this method unrefined oil in glass bottles is exposed for one hour to bright sunlight.
- Aflatoxin is photo-degraded and is reported to lose its toxicity (ikisan.com, 2000).

### 1.1.9. Chemical techniques for aflatoxin reduction

- Aflatoxins can easily be extracted from the contaminated sample through the use of some solvents like acetone and isopropyl alcohol.
- Inorganic solvents like l % sodium bicarbonate or 1 % calcium chloride could effectively remove the toxins up to 80 %. However, these methods also remove some nutritional constituents like proteins.
- This method can be used at the household level since salt is readily available.
- Application of 0.5 % hydrogen peroxide was reported to reduce the aflatoxin levels from 1,000 ppb to 25 ppb.
- Methoxymethane (dimethylether) was reported to be useful in removing aflatoxin from contaminated groundnuts and groundnut meal.
- Some processes like sun-drying, exposure to burning cowdung fumes and spray of 1 % common salt were useful in detoxification of aflatoxins to the levels ranging from 10 to 50 % (ikisan.com, 2000).

### 1.1.10. Foods that contain aflatoxin

A family of potent fungi-produced carcinogens, aflatoxins are commonly found in a variety of agriculture products. Food products contaminated with aflatoxins include cereals (maize, sorghum, pearl millet, rice, wheat), oilseeds (groundnut, soybean, sunflower, cotton), spices (chillies, paprika, black pepper, red pepper, coriander, turmeric, zinger), tree nuts (peanuts, almonds, pistachio, walnuts, coconut), dairy products and dried grape. Several reports of aflatoxin levels in foods in different countries between 1991 and 1998 are given in Table 1.2.

Table 1.2 Reports of aflatoxin in foods (1991-1998) (Pittet, 1998)

| Food Commodity | Country | Year reported | Incidence (%) | Range (μg $kg^{-1}$) |
|---|---|---|---|---|
| Soybeans | Argentina | 1991 | 10 | 1-36 |
| Almonds | USA | 1993 | 1 | Tr-372 |
| Brazil nuts | USA | 1993 | 17 | Tr-619 |
| Dried figs | Austria | 1993 | 13 | 2-350 |
| Nut meg | Japan | 1993 | 43 | 0.2-16.6 |
| Chillies | Pakistan | 1995 | 66 | 1-79.9 |
| Herbs, spices | UK | 1996 | 24 | 1-51 |
| Peanuts | India | 1996 | 45 | 5-833 |
| Maize | Argentina | 1996 | 20 | 5-560 |
| Pistachio nuts | Netherlands | 1996 | 59 | 2-165 |
| Wheat | Uruguay | 1996 | 20 | 2-20 |
| Maize | India | 1997 | 47 | 5-666 |
| Rice | Equador | 1997 | 9 | 6.8-40 |
| Peanuts | Brazil | 1998 | 51 | 43-1099 |
| Spices | Sweden | 1998 | 90 | 0.1-62 |

## 1.2. Red Pepper

The *Capsicum* genus, which is a member of the *Solanaceae* family, represents a diverse plant group, from the well known sweet green bell pepper to the fiery hot, recently exploited habanero. The genus *Capsicum* consists of approximately 22 wild species and five domesticated species: *C. annuum, C. baccatum, C. chinense, C. frutescens, and C. pubescens* (Bosland, 1994). Red pepper (Appendix Figure B.3) refers primarily to *Capsicum annuum L. (Linnaeus)* and *Capsicum frutescens L.* plants which are used in the manufacture of selected commercial products known for their pungency and color. Chile fruits are considered vegetables, but are berries botanically.

Red pepper, *Capsicum*, has been domesticated for 7,000 years. It has been a part of the human diet since about 7500 BC (MacNeish, 1964). The *Capsicum* genus,

chile, of which origin is South and Middle America, is historically associated with the voyage of Columbus (Heiser, 1976). Columbus is given credit for introducing chile to Europe, and subsequently to Africa and to Asia.

On his first voyage, he encountered a plant whose fruit mimicked the pungency of the black pepper; *Piper nigrum L.* Columbus called it red pepper because the pods were red. Capsicum is not related to the *Piper* genus. Anghiera (1493) wrote that Columbus brought home "pepper more pungent than that from the Caucasus."

Chile spread rapidly across Europe into India, China, and Japan. The new spice, unlike most of the solanums from the Western Hemisphere, was incorporated into the cuisines instantaneously. Probably for the first time, pepper was no longer a luxury spice only the rich could afford. Since its discovery by Columbus, chile has been incorporated into most of the world's cuisines. It has been commercially grown in the United States since at least 1600, when Spanish colonists planted seeds and grew chile using irrigation from the Rio Chama in northern New Mexico (DeWitt and Gerlach, 1990).

The reported life zone for capsicum peppers is 7 to 29 °C with an annual precipitation of 0.3 to 4.6 m and a soil pH of 4.3 to 8.7. *Capsicum* species are cold sensitive and generally grow best in well-drained, sandy or silt-loam soil. Plantings are established by seeding or transplanting. Flowering usually occurs three months after planting. Hot and dry weather is desirable for fruit ripening. Fruit is generally handpicked as it ripens, and then allowed to dry in the sun, although artificial drying is often employed in Europe and the U.S. (Simon et al., 1984).

Red pepper is an excellent product in terms of nutritional value (Table 1.3). The color of red pepper comes from carotenoids like capsanthin, carotene, capsorubin, kryptoxantin, capsaisinoid, zeaxantine and lutein (Erdoğrul, 2000). Typical aroma of red pepper comes from many specific esters and pyrasine, thiazole and alcohol compounds.

Table 1.3 Chemical composition of dry hot red pepper (Duman, 2001)

| Composition of dry hot red pepper | Conc/100 g |
|---|---|
| Energy | 318 kcal |
| Water | 8.1 g |
| Protein | 12.0 g |
| Lipid | 17.3 g |
| Carbohydrates | 56.6 g |
| Fiber | 24.9 g |
| Ash | 6.0 g |
| Ca | 148.0 mg |
| Fe | 8.0 mg |
| P | 293.0 mg |
| K | 2014.0 mg |
| Mg | 152.0 mg |
| Na | 30.0 mg |
| Zn | 2.0 mg |
| Vitamin C | 76.0 mg |
| Vitamin A | 41610 IU |

Red pepper is recognized as a high risk product with regard to the production of aflatoxin. In the south east region of Turkey, red pepper which is produced and consumed largely is generally processed under unhygienic conditions. Consequently, the risk of microbial contamination is high. Although there have not been wide cases caused by aflatoxin intake through pepper consumption, some amounts of peppers have been reported to contain aflatoxin. Among the pepper-producer countries in the world (Table 1.4), Turkey has an important place with 1,080, 000 tones pepper production per annum, of which 69,000 tones was achieved in GAP (South East Anatolian Project) region. Pepper is mostly grown in and distributed to other regions and exported to many European countries from Southeastern region of Turkey.

Table 1.4 Top ten pepper producing countries and total pepper production of the world in 2000 (Duman, 2001)

| Producing Country | Production Amount (Ton) |
|---|---|
| China | 7,683,127 |
| Mexico | 1,813,252 |
| Turkey | 1,390,000 |
| Spain | 936,300 |
| Nigeria | 715,000 |
| U.S.A. | 694,950 |
| Ecuador | 600,000 |
| Indonesia | 496,908 |
| Italy | 316,209 |
| Korea | 307,000 |
| World | 18,500,622 |

In the industrial production of red pepper, drying is achieved on pulsating trays in a hot air circulated tunnel at temperatures between 40-90 °C for 1-12 hours. The drying process should remove about 90 % of the moisture from the peppers. Agricultural products standard and Turkish Standard (TS 2419) for moisture content in red pepper are 15 and 11 % (wb), respectively. Traditionally, hot red pepper is kept under sun in polyethylene bags during day time to perspire and is kept in cloth bags throughout the night for 10-12 days. The optimum temperature for drying red pepper to prevent the formation of aflatoxin while maintaining good sensory quality was found to be 65 °C (Duman, 2001).

European Union (EU) and Turkish Food Nutriment Codex set limits of 10 ppb for total aflatoxin and 5 ppb for aflatoxin $B_1$ in red pepper. The FDA has set limits of 20.0 ppb for total aflatoxin and 10.0 ppb for aflatoxin $B_1$ in all foods except milk, which has a limit of 0.5 ppb ($M_1$).

### 1.3. High Hydrostatic Pressure (HHP)

High pressure treatment has been known as a potential preservation technique for foods and food ingredients for over a century and the recent commercial success of the process in Japan has stimulated significant research activity in this area in many other countries. The scientific basis for the success of the process does involve many disciplines including both chemistry and microbiology.

However, its commercial success, as with many food operations, has to be based on effective integration with the engineering disciplines involved in the design and manufacture of equipment and plant capable of efficiently and safely applying such pressure to both solid and liquid foods. That the effect of pressure on food systems may lead to various chemical and biological changes without bringing out the same chemical changes that heat treatment does is seen to be a major advantage of the technology (Ledward et al., 1995).

#### 1.3.1. Effects of HHP on foods

High pressure is effective on reactions of importance in food systems. Any reaction, conformational change or phase transition that is accompanied by a decrease in volume will be encouraged at high pressures whereas reactions involving an increase in volume will be inhibited (Ledward et al., 1995). Vegetative cells are inactivated (Figure 1.3) at ca. 3,000 bars at ambient temperature, while spore inactivation requires much higher pressures (6,000 bars or more) in a combination with a temperature rise to 60-70 °C. Certain enzymes are inactivated at 3,000 bars, while others are very difficult to inactivate at all within the pressure range that is practically available today. Moisture level is extremely important in this context, little effect being noticeable below 40 % moisture content (Ohlsson and Bengtsson, 2002). Safety requirements for critical control factors (HHP) are given as follows:

- Greater pressure and longer time of application may cause changes in the appearance of selected foods. Raw high protein foods and structurally fragile foods (like strawberries) may be physically altered.
- There is an increase of 3 °C for each 100 MPa of the product through adiabatic heating, depending on the composition of the food. Therefore to achieve a uniform process temperature, uniform initial temperature is required.
- As pressure decreases the volume of the food and equal expansion occurs on decompression, therefore packaging material should be able to accommodate up to 15 % reduction in volume, so that seal integrity and barrier properties are conserved (Package integrity) (Alpas, 2005).

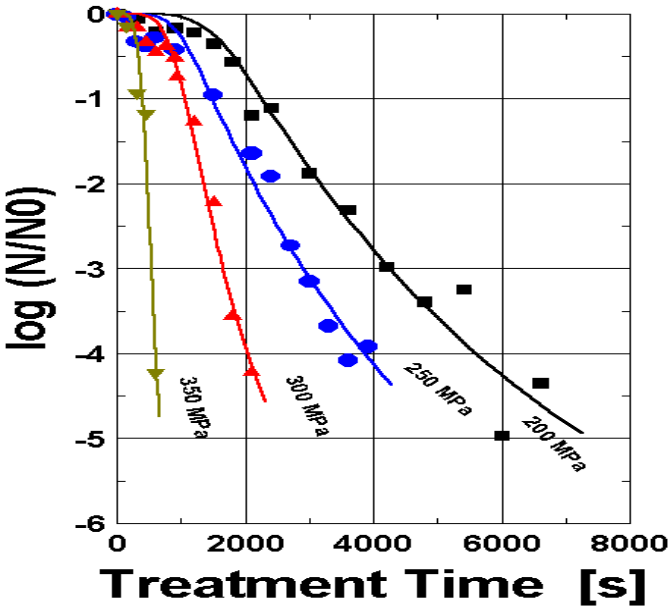


Figure 1.3 Inactivation of a Listeria strain in meat product by high pressure (Alpas, 2005)

### 1.3.2. Utilizations of HHP in food industry

High pressure processing (HPP), also described as high hydrostatic pressure (HHP), or ultra high pressure (UHP) processing, subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa. Process temperature during pressure treatment can be specified from below 0 °C (to minimize any effects of adiabatic heat) to above 100 °C. Vessels (Appendix Figure B.4) are uniquely designed to safely withstand these pressures over many cycles. Commercial exposure times at pressure can range from a millisecond pulse (obtained by oscillating pumps) to a treatment time of over 1200 s (20 min) (Ledward et al., 1995).

In contrast to thermal processing, economic requirements for throughput may limit practical exposure times to less than 20 min. Pressures used in the HPP of foods appear to have little effect on covalent bonds (Tauscher, 1999); thus, foods subjected to HPP treatment at or near room temperature will not undergo significant chemical transformations due to the pressure treatment itself. Chemical changes in the food generally will be a function of the process temperature and time selected in conjunction with the pressure treatment. Some of the current applications of HPP in the food industry are shown in Table 1.5.

Table 1.5 Current applications of high pressure processing (Ohlsson and Bengtsson, 2002)

| Product | Manufacturer | Process Conditions |
|---|---|---|
| Jams, fruit dressing, fruit sauce topping, yoghurt fruit jelly | Meidi-ya Company, Japan | 400 MPa, 10-30 min, 20 °C |
| Grapefruit juice | Pokka Corp., Japan | 120-400 MPa, 2-20 min, 20 °C + additional heat treatment |
| Mandarin juice | Wakayama Food Ind., Japan | 300-400 MPa, 2-3 min, 20 °C |
| Non-frozen tropical fruits | Nishin Oil Mills, Japan | 50°C -200 MPa |
| Tenderised beef | Fuji Ciku Mutterham, Japan | 100-50 MPa, 30-40 min, 20 °C |
| Avocado juice | Avomex, USA | 700 MPa, 600-800 L/h |
| Orange juice | UltiFruit, France | 500 MPa, 5 or 10 min cyles, included a 1 min hold |

### 1.3.3. Advantages and disadvantages of HHP

Because of the complexity of reactions that can take place in a food system it is not surprising that it is difficult to predict the effect of high pressure on any particular system. The advantages of HHP are listed below:

- The HHP system is energy efficient, with minimal sanitation problem.
- The antimicrobial effect is isostatic and instantaneous.
- Pressure transmission is not controlled by product size and no edge or thickness effect takes place.
- effectively destroys vegetative cells, enzymes, yeast and molds. Semi-effective on spores.
- does not adversely effect the food quality.

- heat-labile nutrients and natural flavors are effectively retained.
- no additives are required.
- increased shelf-life
- lower cost (maintenance)
- new formulations possible (Alpas, 2005).

On the other hand, the major commercial limitations of hydrostatic pressure preservation of foods are that it is a batch process (as opposed to continuous process) and at higher pressure ranges the equipment is very costly (capital) and less durable owing to metal fatigue. At high pressure ranges the natural texture and color of some foods, such as meat, can also change, reducing their acceptance. However, these disadvantages can be overcome by using lower pressure and shorter time by applying moderately higher temperatures (40, 50 °C) (Mertens & Deplace 1993). Limited studies have reported that the loss of viability of bacterial cells is greatly enhanced with pressurization at moderately higher temperatures (Ludwig et al. 1992).

# CHAPTER 2

# MATERIALS AND METHODS

## 2.1. Materials

Contaminated dried red pepper samples which had been produced by some of red pepper producers in Gaziantep were provided from Agriculture Control Laboratory, Gaziantep where the samples had been stored after their HPLC analyses. 3 different samples were arranged, because it was difficult to find contaminated samples in a short time period and the high hydrostatic pressure units had restricted uses over time. The first one (1,150 kg - 32,07 ppb total afla.) was used for pressurizing at 150 and 200 MPa, the second (1,000 kg - 79.05 ppb total afla.) at 250 MPa and the third one (1,900 kg - 93.44 ppb total afla.) at 300 and 350 MPa. As the reagents methanol (reagent-grade), methanol (HPLC grade) and asetonitril (reagent-grade) were used, which were provided from Merck, Germany.

## 2.2. Experimental Set-up

In this study, the combined effect of high hydrostatic pressure and temperature on aflatoxin content of dried and powdered red pepper was investigated. As the pressurizing units, two different hydrostatic pressure vessels were used: The first one (Figure 2.1) was installed in the workshop of Civil Engineering Department, University of Gaziantep, which could reach high pressures up to 350 MPa and the second one (Figure 2.2 and Appendix Figure B.5) is in the Food Engineering Department, Middle East Technical University (METU) which could reach high pressures up to 250 MPa. The former was used for at 300 and 350 MPa and the latter at 150, 200, and 250 MPa. For the aflatoxin analyses, HPLC units in the Agricultural Control Laboratory and Trade Stock-Exchange Laboratory in Gaziantep were utilized. Both machines worked in the same manner.

Each of the red pepper specimens was ground in a mill (Thomas-WILEY, USA) and then mixed in a mixer (Silverson, UK) for 20 minutes to have a homogeneous sample. Moisture contents of the samples were determined by entrainment method (TS 2134) and found to be around 21 % (wb), 22 % (wb), and 26 % (wb), respectively.

### 2.3. Application of High Hydrostatic Pressure

A total of 131 samples were used. For each experiment, dublicates were run except for the pressure 150 MPa (no dublicate). The samples (30 g) were placed into 3 fold dry polyethylene based stretch films (Koroplast, Turkey) (200 mm x 200 mm x 10 mic) which were previously immersed into $H_2O_2$ solution (20 g/kg) and dried. After evacuating the films, the ends of the films were double clipped. The samples were then submerged into the cylinders (Figure 2.1 and Figure 2.2) filled totally with distilled water and compressed at each of 150, 200, 250, 300 and 350 MPa for 4, 6, 8, 10, and 12 minutes. The experiments were carried out at 20, 30, and 40 ± 2 ˚C except for 150 MPa which was only accompanied by 40± 2 ˚C. After pressurizing, samples were stored at -20 °C until HPLC analyses.

### 2.4. Design of Hydrostatic Pressure Vessels

The hydrostatic pressure unit in the University of Gaziantep is 170 mm high, 150 mm in diameter with two holes, having diameters of 40 mm at the top and 100 mm at the bottom, respectively. The top hole is used for hydrostatic pressure and the bottom hole for suspending the sample. Typical cylinder capacity is 150 ml.

When the hydrostatic pressure cylinder was designed, the first model was prepared from stainless steel which was processed according to the required sizes to apply the pressure from the pressure unit at the laboratory of Mechanical Engineering Department, University of Gaziantep. The inner surface of the hydrostatic cylinder unit was honed in the Organised Industrial Area, Gaziantep. The piston was also made of stainless steel, containing three “O” rings and one nut-ring. The high pressure was supplied by a pressure generator (MFL universal system, Pruf and Mess, Germany). Details of the equipment are given in Figure 2.1.

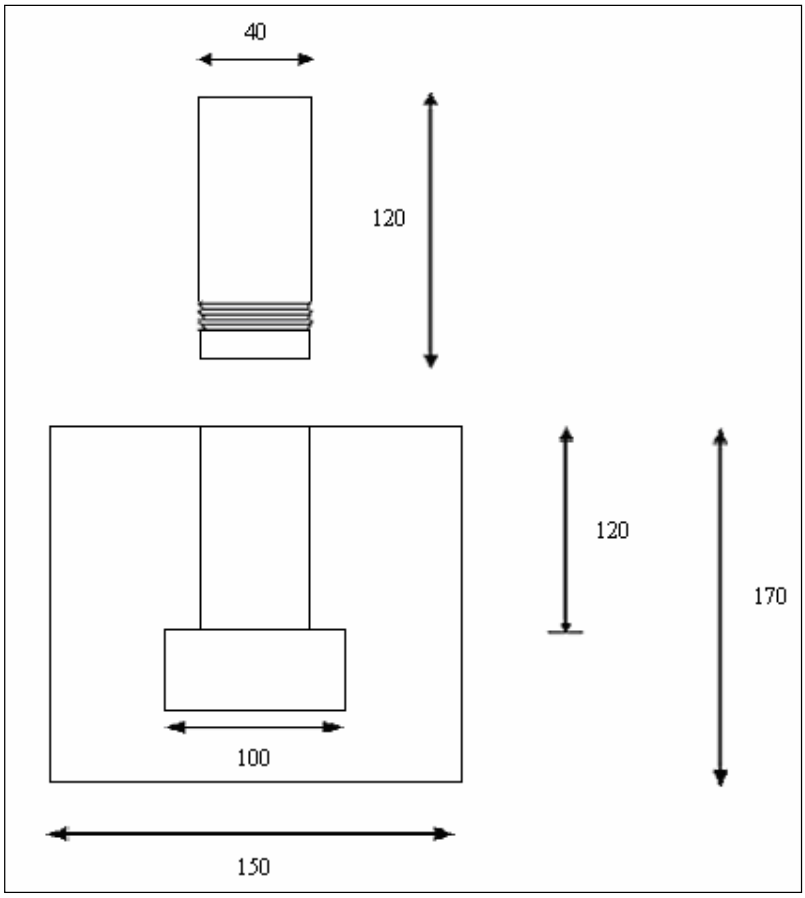


Figure 2.1 Hydrostatic pressure cylinder, University of Gaziantep (Karataş and Ahi, 1992)

The second high pressure vessel was constructed in the Electrical & Electronical Engineering Department, METU. It had a built-in heating system that could adjust the temperature of the water in the pressure chamber between 20 and 90 ˚C and a pressure controlling device. The reservoir capacity is ca. 13 ml. As the pressurizing liquid water was used. The details of the HHP unit are given in Figure 2.2 and Appendix Figure B.5.

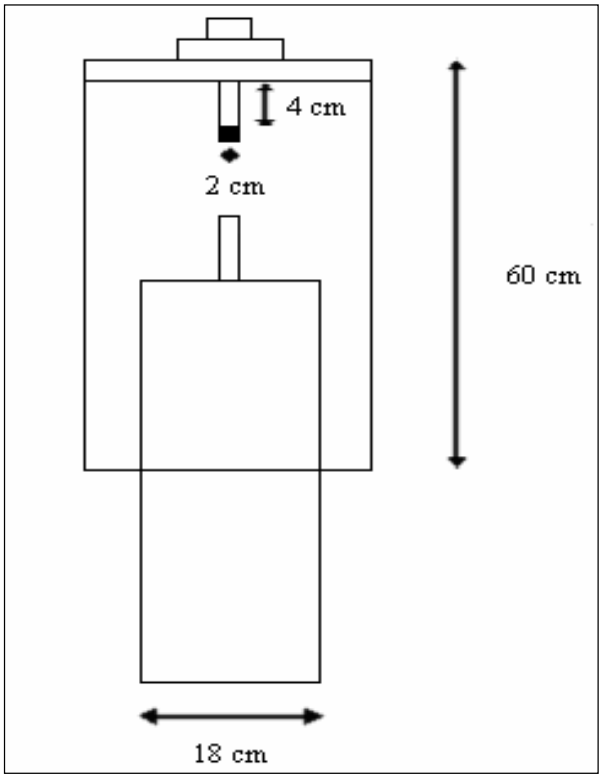


Figure 2.2 Hydrostatic pressure vessel, METU

### 2.5. Aflatoxin Analysis by HPLC

Before the samples are given to the HPLC unit, it was confirmed by the calibration with "ready to use aflatoxin standard solutions" for $B_1$, $B_2$, $G_1$, and $G_2$ types (R-Biopharm Rhone, Scotland) that correct measurements were done (Aflaprep IFU, P07v13). So accuracy was assured.

#### 2.5.1. Extraction of aflatoxin

Firstly, 25 g sample and 5 g salt (NaCl) were mixed with 100 ml methanol-water mixture (80:20) and shaken for 30 minutes in a mechanical shaker (Yellowline, Germany). Then, the mixture was filtered through Whatman No. 4 filter paper. The filtrate was used as extract for further analysis (Aflaprep IFU, p07v13).

### 2.5.2. Determination of aflatoxin content

40 ml double distilled water was added to 10 ml of extract and mixed well. Then, 10 ml of this diluted extract was passed into an injector by the use of microfiber filter (GF/D Whatman, England).

Following this, the extract in the injector was passed through the immunoaffinity column (Vicam, USA) at a flow rate of 2-3 ml/min by the use of microfiber filter. Before letting the column dry, 20 ml double distilled water was passed through the column at 2-3 ml/min. Following this, 1 ml methanol (HPLC grade) was passed through the column at 2-3 ml/min and the eluant was collected into a clean tube. Then, 1 ml double distilled water was passed through the column into the tube. The tube was shaken in the shaker for a few minutes. Finally, 2 ml was taken into the small vial and given to the HPLC unit (Hewlett Packard, Agilent 1100, USA) (Appendix Figure B.6) to be analysed.

ODS-2 (octadecylsilicate-2), C18 column (4.6 mm x 250 mm; 5µm) was used with a mobile phase (double distilled water-methanol-asetonitril (600:300:200)). The flow rate was 1 ml/min. The excitation and emission of fluorescence detector were at 360 nm and 440 nm. Kobra cell bromine derivatization system was used as the post-column (Aflaprep IFU, p07v13). Finally, the amounts of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$, and $G_2$ were recorded.

## 2.6. Kinetic Evaluation of the Experiments

In order to evaluate the kinetic behavior of the possible reactions which were observed during the experiments, the integrated form of rate law (extended for zero-order reactions) and Arrhenius expressions were selected. The details of the methods are given below at sections 2.6.1. and 2.6.2. All graphs in this thesis were prepared by using SigmaPlot 2000 (version 6.0) program.

### 2.6.1. Determining the order of a reaction with the integrated form of rate laws

The integrated form of the rate laws for zero-, first- and second-order reactions provides a way of determining the order of a reaction. We can start by assuming, for the sake of argument, that the reaction is zero-order in reactant A.

If a reactant is said to be of 0 order in a reaction, it does not effect the rate of the reaction. Then, the reaction rate equation becomes;

$$-\frac{d[A]}{dt} = k \tag{2.1}$$

which on integration of both sides gives;

$$[A] = -kt + C \tag{2.2}$$

When t = 0, the concentration of A is $[A]_0$. The constant of integration must be $[A]_0$.

Now the integrated form of zero-order kinetics can be written as follows;

$$[A] = [A]_0 - kt \tag{2.3}$$

If the reaction is zero order, plotting [A] versus t will give a straight line with slope -k. The rate of reaction at any particular time is found as the gradient of the concentration vs. time graph. The gradient of rate of reaction vs. concentration is zero as the reactant has no effect on the overall reaction. So graphical plots of concentration vs. time and rate vs. time would look like Figure 2.3 and Figure 2.4, respectively.

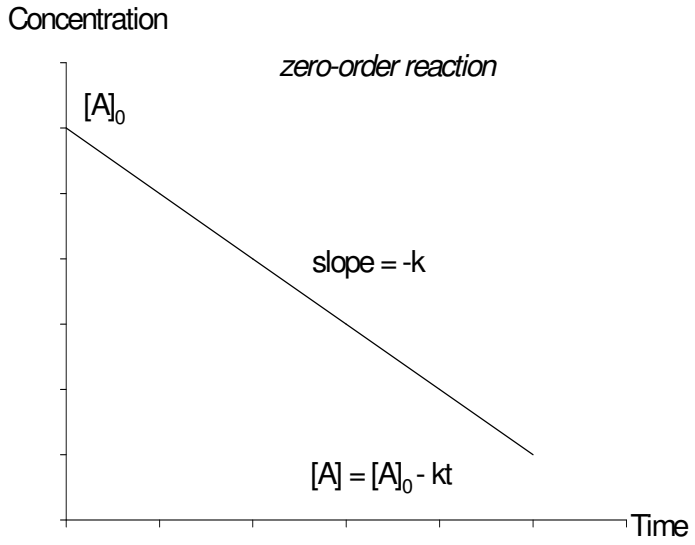


Figure 2.3 Concentration vs. time (zero-order reaction)

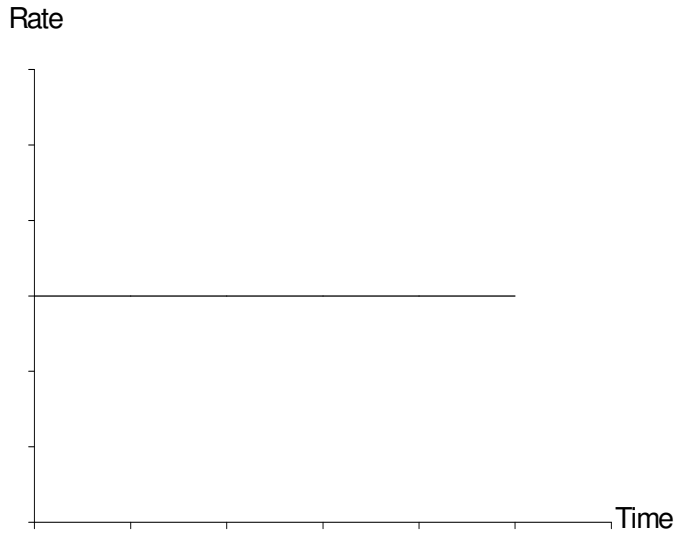


Figure 2.4 Reaction rate vs. time (zero-order reaction)

If the reaction is first-order; the rate is proportional to the concentration of a single reactant raised to the first power;

$$-\frac{d[A]}{dt} = k[A] \tag{2.4}$$

$$-\frac{d[A]}{[A]} = kdt \tag{2.5}$$

Equation 2.5 represents the differential form of the rate law. Integration of this equation and determination of the integration constant C produces the corresponding integrated law.

Integrating Equation 2.5 yields:

$$\ln [A] = -kt + C \tag{2.6}$$

The constant of integration C can be evaluated by using boundary conditions. When t = 0, $[A] = [A]_0$. $[A]_0$ is the original concentration of A. Substituting into Equation 2.6 gives:

$$C = \ln [A]_0 \tag{2.7}$$

$$\ln \frac{[A]}{[A]_0} = -kt \tag{2.8}$$

Equation 2.8 can also be written as:

$$\ln [A] = \ln [A]_0 - kt \tag{2.9}$$

If the reaction is first-order in A, a plot of the natural logarithm of the concentration of A versus time will be a straight line with a slope equal to -k, as shown in Figure 2.5.

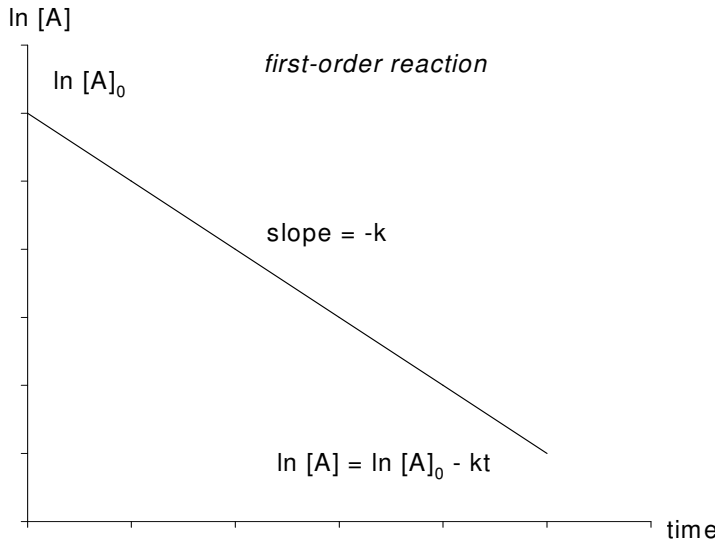


Figure 2.5 ln [A] vs. time (first-order reaction)

If the reaction is not zero or first-order in A, we assume that it is second-order in A.

$$-\frac{d[A]}{dt} = k[A]^2 \tag{2.10}$$

Separating the variables;

$$-\frac{d[A]}{[A]^2} = kdt \tag{2.11}$$

and integrating;

$$\frac{1}{[A]} = kt + C \tag{2.12}$$

Provided that $[A] = [A]_0$ at $t = 0$ the constant of integration C becomes equal to $1/[A]_0$.

Thus the second order integrated rate equation is

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt \tag{2.13}$$

If the reaction is second-order in A, a plot of the reciprocal of the concentration of A versus time will be a straight line with a slope equal to k, as shown in Figure 2.6 (purdue.edu, 2006).

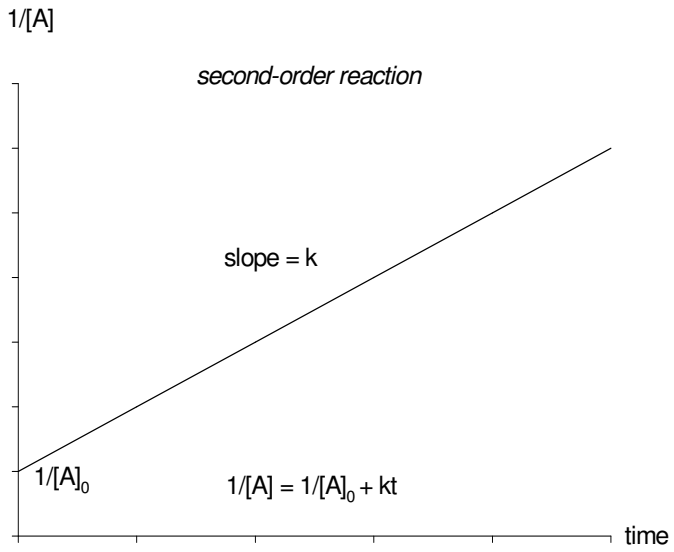


Figure 2.6 1/[A] vs. time (second-order reaction)

In order to determine the order of the reaction which involves the decrease in aflatoxin; the graphs ([A] vs. t), (ln [A] vs. t), and (1/[A] vs. t) were plotted at each of pressure-temperature combinations. The more linear the graphs ([A] vs. t), (ln [A] vs. t), and (1/[A] vs. t), the more likely the reaction order be zero, first or second, respectively.

### 2.6.2. Evaluation of the activation energy by Arrhenius expression

The theory of the temperature effect on the reaction rate originated from the temperature effect on the equilibrium constant. It is known that:

$$\frac{d\left[\ln K\right]}{d\left[\frac{1}{T}\right]} = -\frac{H}{R} \tag{2.14}$$

where K is an equilibrium constant, R is the gas constant and H is the heat of reaction. Since the equilibrium constant $K= k_1/k_2$, $k_1$ and $k_2$ are the rate constants for

the forward and reverse reactions, respectively. Substituting the relationship into Equation 2.14, we obtain:

$$\frac{d(\ln k_1)}{d\left[\frac{1}{T}\right]} - \frac{d(\ln k_2)}{d\left[\frac{1}{T}\right]} = -\frac{H}{R} \tag{2.15}$$

Arrhenius (1889) recognized that Equation 2.15 could be conveniently divided into two parts, each having the form of:

$$\frac{d(\ln k)}{d\left[\frac{1}{T}\right]} = -\frac{E_a}{R} \tag{2.16}$$

where $E_a$ is referred by Arrhenius as representing the energy difference between the reactants and an activated species. The term $E_a$ is therefore called the activation energy.

Taking $E_a$ as a constant, Equation 2.16 can be integrated to yield:

$$\ln k = \ln A - \frac{E_a}{RT} \tag{2.17}$$

where ln A is the constant of integration. Equation 2.17 can be converted to:

$$k = Ae^{\left[\frac{-E_a}{RT}\right]} \tag{2.18}$$

which is the most widely adopted form of the Arrhenius equation. The term A is the preexponential factor. This is related to the frequency of molecular collisions in the collision theory and to the entropy term in the transition state theory.

The activation energy is the energy barrier that the reactants must surmount in order to react. Therefore the activation energy is viewed as an energetic threshold for a fruitful reaction.

Application of Arrhenius equation in pharmaceutical stability testing is straightforward. In the isothermal method, the system to be investigated is stored under several high temperatures with all other conditions fixed. Excess thermal exposure accelerates the degradation and thus allows the rate constants to be determined in a shorter time period.

In order to find the activation energy ($E_a$) for a reaction; the rate constant at each temperature is determined. The logarithm of the rate constant is linearly related to 1/T as shown in Equation 2.17. Although it is possible to calculate A and $E_a$ with Arrhenius equation by just using rate constants at two temperatures, it would be more realistic and reliable to use at least three rate constants (preferably more) at three different temperatures and determine A and $E_a$.

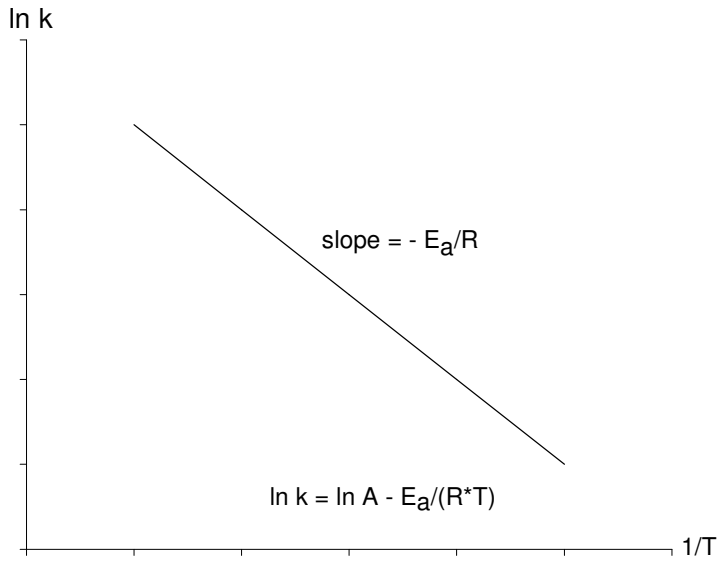


Figure 2.7 Arrhenius Plot for the evaluation of activation energy

Having determined the values of A and E, it is then possible to use Arrhenius equation to project the rate constant at any temperature. This process is graphically represented in Figure 2.7. Arrhenius equation is probably one of the most important tools that a formulator possesses in the product development process (ScienTek Software, 2003).

# CHAPTER 3

# RESULTS AND DISCUSSION

## 3.1. High Hydrostatic Pressure Application at Different Temperatures

### 3.1.1. Effect of high hydrostatic pressure at 20 °C

The results of these experimental runs are illustrated in Figure 3.1 and Appendix Table A.1. The percent reductions in total aflatoxin in powdered red pepper at high hydrostatic pressures of 200, 250, 300, and 350 MPa for 4 minutes were about 5, 10, 16, and 22 %; and for aflatoxin $B_1$, 6, 10, 17, and 23 %, respectively. Accordingly, the same percentage reductions were observed for total aflatoxin and $B_1$ at 250 MPa, and the reduction in aflatoxin $B_1$ was 1.2, 1.06, and 1.05 times higher than that of total aflatoxin under 200, 300, and 350 MPa, respectively. For aflatoxin $B_2$, $G_1$ and $G_2$, there were both increases and decreases in the concentrations with pressurizing. Such as, there were increases in $B_2$ (6 % and 9 %) between 250 and 300 MPa and 300 and 350 MPa, respectively, while $G_1$ concentration was higher at each high hydrostatic pressure than the original, reaching its maximum (151 % of its original value) at 200 MPa. Finally, there was a 2 % increase in the concentration of $G_2$ between 250 and 300 MPa.

As Figure 3.2 and Appendix Table A.2 indicate, the percent reductions in total aflatoxin at high hydrostatic pressures of 200, 250, 300, and 350 MPa for 6 minutes were about 11, 11, 18, and 23 %; and for aflatoxin $B_1$, 11, 11, 19, and 25 %, respectively. According to these results, the reductions in total aflatoxin and $B_1$ almost coincide, $B_1$ reduction being the same as the total aflatoxin reduction at 200 and 250 MPa; and 1.06 and 1.09 times higher than the total aflatoxin reduction at 300 and 350 MPa, respectively. For $B_2$, there were concentration increases of 5 % between 250 and 300 MPa and 34 % between 300 and 350 MPa. For $G_1$, there were two concentration increases (each of 12 %) at 200 MPa and between 300 and 350 MPa. There was also a high concentration increase (40 %) for $G_2$ which was observed between 250 and 300 MPa.

As it can be followed from Figure 3.3 and Appendix Table A.3, pressurizing at 200, 250, 300, and 350 MPa (for 8 minutes), the percent reductions in total aflatoxin were about 16, 18, 24, and 28 %; and in aflatoxin $B_1$, 16, 19, 24, and 28 %, respectively. It was observed that the reduction in $B_1$ was approximately the same as total aflatoxin reduction at 200, 300, and 350 MPa; and 1.06 times higher than total aflatoxin reduction at 250 MPa. Meanwhile, $B_2$ showed an increase of 22 % between 200 and 250 MPa and 8 % between 300 and 350 MPa, respectively. Moreover, $G_1$ concentration increased at 200 MPa (24 %) and between 250 and 300 MPa (18 %). Finally, the increase in the concentration of $G_2$ was observed between 300 and 350 MPa as 11 %.

Moreover, the percentages of reduction in total aflatoxin for 10 minutes at 200, 250, 300 and 350 MPa were 28, 33, 36, and 39 %; and in aflatoxin $B_1$, 29, 34, 37, and 40 %, respectively. It was observed that the reduction in $B_1$ was 1.04, 1,03, 1.03, and 1,03 times higher than the reduction in total aflatoxin at 200, 250, 300, and 350 MPa, respectively. The concentration of $B_2$ increased 11 % during the pressure application between 300 and 350 MPa. The concentration increase of $G_1$ was observed between 250 and 300 MPa as 25 %, while amount of $G_2$ increased by 30 % between 300 and 350 MPa (Figure 3.4 and Appendix Table A.4).

Finally, for 12 minutes at 200, 250, 300, and 350 MPa, the reductions in total aflatoxin were 31, 34, 39, and 49 %; and in aflatoxin $B_1$, 31, 36, 39, and 49 %, respectively. It was observed that the reduction in $B_1$ was the same as total aflatoxin reduction at 200,300, and 350 MPa; and 1.06 times higher than the reduction in total aflatoxin at 250 MPa. The concentration of $B_2$ showed an increase by 30 % between 200 and 250 MPa, while $G_1$ increased by 7 % between 250 and 300 MPa and $G_2$ increased by 16 % between 300 and 350 MPa. (Figure 3.5 and Appendix Table A.5)

This indicates that the reduction rate is a direct function of pressure and time for the total aflatoxin and aflatoxin $B_1$ destruction, which almost coincide with each other. Different pressures and time periods caused fluctuations in the concentrations of aflatoxin $B_2$, $G_1$ and $G_2$. Some of $B_1$ may have changed to the chemical conformation of $B_2$, $G_1$, and $G_2$, which caused their concentration to increase. As the results indicate, the maximum percent reduction at 20 °C in total aflatoxin and $B_1$

were observed as 49 % at 12$^{th}$ minute. This amount of reduction may be considered small if it is compared to aconitase (ACO), a metabolic enzyme produced by three strains of Listeria monocytogenes, which was completely inactivated after exposure to pressures of between 150 and 200 MPa for 15 minutes at ambient temperature (Simpson and Gilmour, 1997).

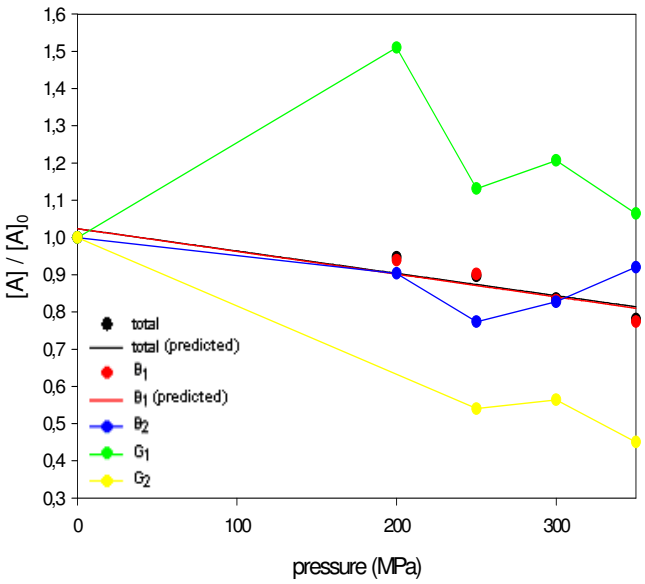


Figure 3.1 Changes in total aflatoxin ($R^2$ = 0.90), aflatoxin $B_1$ ($R^2$ = 0.90), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 4 min with various pressures at 20 °C

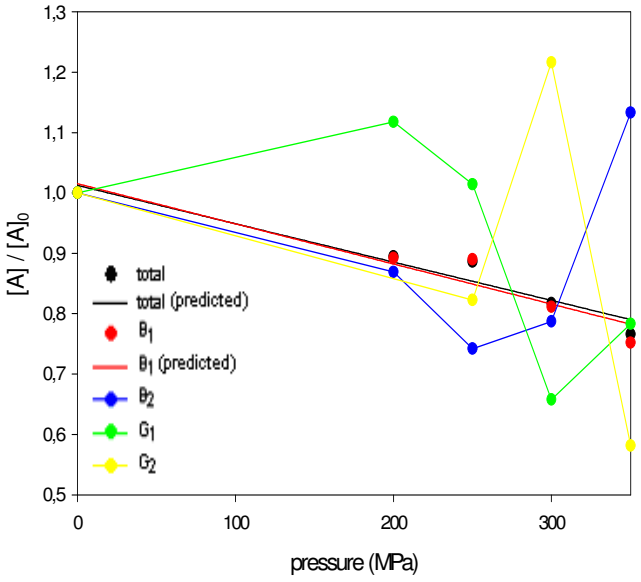


Figure 3.2 Changes in total aflatoxin ($R^2$ = 0.94), aflatoxin $B_1$ ($R^2$ = 0.92), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 6 min with various pressures at 20 °C

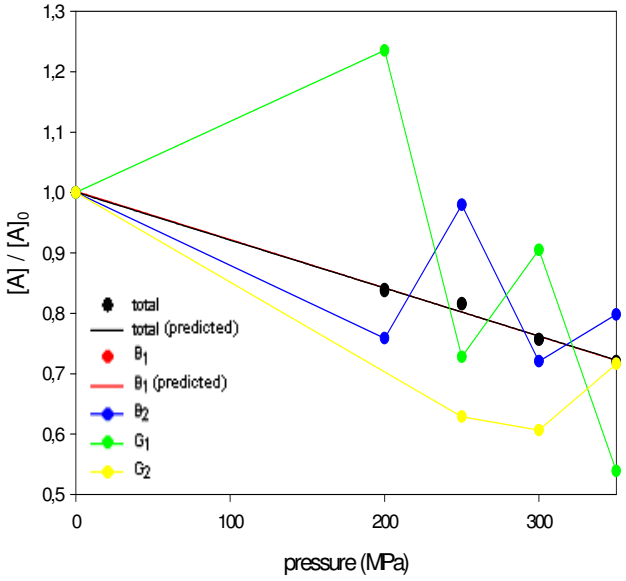


Figure 3.3 Changes in total aflatoxin ($R^2$ = 0.99), aflatoxin $B_1$ ($R^2$ = 1), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 8 min with various pressures at 20 °C

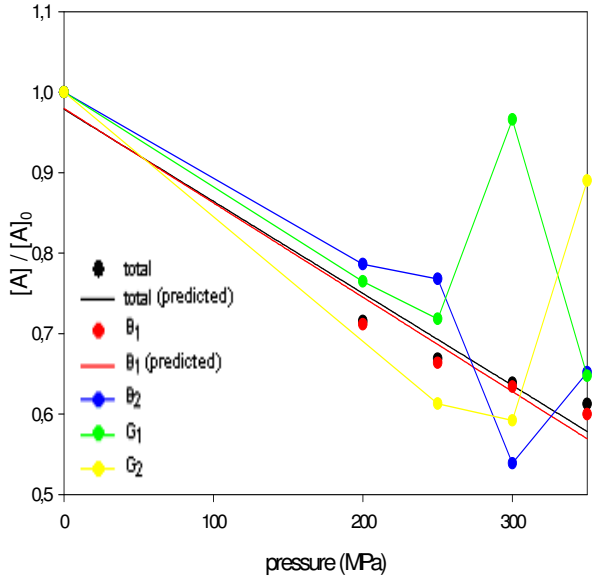


Figure 3.4 Changes in total aflatoxin ($R^2$ = 0.97), aflatoxin $B_1$ ($R^2$ = 0.97), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 10 min with various pressures at 20 ℃

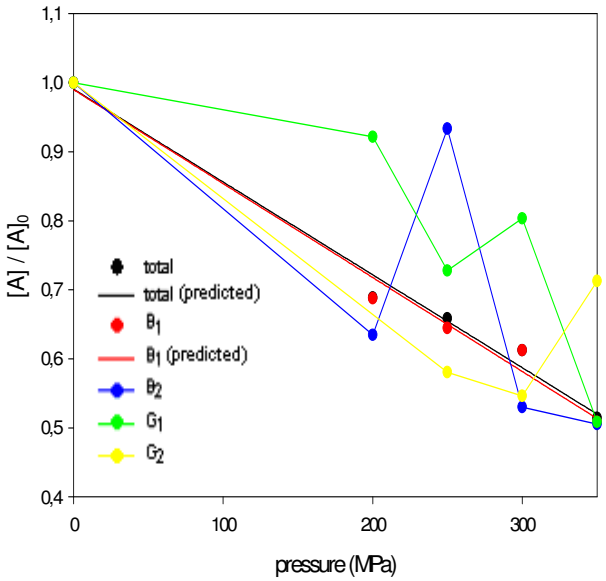


Figure 3.5 Changes in total aflatoxin ($R^2$ = 0.99), aflatoxin $B_1$ ($R^2$ = 0.99), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 12 min with various pressures at 20 ℃

### 3.1.2. Effect of high hydrostatic pressure at 30 °C

As Figure 3.6 and Appendix Table A.6 illustrate, the percentages of reduction in total aflatoxin at high hydrostatic pressures of 200, 250, 300, and 350 MPa for 4 minutes were about 6, 13, 18, and 23 %; and in aflatoxin $B_1$, 8, 13, 19, and 23 %, respectively. It was observed that the reduction in $B_1$ for 4 minutes was the same as total aflatoxin reduction at 250 and 350 MPa; and 1.33 and 1.06 times higher than the reduction in total aflatoxin at 200 and 300 MPa, respectively. There was an increase of 6 % in the concentration of $B_2$ at 200 MPa and 23 % between 250 and 300 MPa. Again, the concentration of $G_1$ increased at 200 MPa and between 300 and 350 MPa by 65 % and 22 %, respectively. Finally, pressurizing between 250 and 300 MPa caused the concentration of $G_2$ to increase by 2 %.

It was observed through this study that the reductions caused at pressures of 200, 250, 300 and 350 MPa (6 min) in total aflatoxin were about 11, 14, 19, and 27 %; and in aflatoxin $B_1$, 11, 14, 19, and 29 %, respectively. Accordingly, the reduction in $B_1$ under pressure of 200, 250, and 300 MPa was the same as that in total aflatoxin; and 1.07 times higher than the reduction in total aflatoxin at 350 MPa. In the meantime, there was 15 % increase in the concentration of $B_2$ between 200 and 250 MPa and 19 % between 300 and 350 MPa, while $G_1$ concentration showed an increase of 6 and 4 % at 200 and between 300 and 350 MPa, respectively. Finally, $G_2$ concentration rose by 2 % between 200 and 250 MPa (Figure 3.7 and Appendix Table A.7).

As demonstrated in Figure 3.8 and Appendix Table A.8, the percentages of reduction in total aflatoxin at 200, 250, 300, and 350 MPa (for 8 min) were about 16, 21, 26, and 29 %; and in aflatoxin $B_1$, 17, 22, 27, and 30 %, respectively. It was observed that the reduction in $B_1$ for 8 minutes was 1.06, 1.05, 1.04, and 1.03 times higher than the reduction in total aflatoxin at 200, 250, 300, and 350 MPa, respectively. There were again increases of concentration in $B_2$, which were 1 and 27 % at 200 and between 250 and 300 MPa, respectively. While $G_1$ concentration showed an increase of 38 % between 300 and 350 MPa, $G_2$ concentration rose by 58 % until 250 MPa and by 13 % between 300 and 350 MPa, respectively.

Furthermore, the percent reductions in total aflatoxin for 10 minutes at 200, 250, 300, and 350 MPa were 29, 34, 38, and 40 %; and in aflatoxin $B_1$; 30, 36, 38, and 41 %, respectively. According to these results, reduction in $B_1$ was the same as that in total aflatoxin at 300 MPa; and 1.03, 1.06, and 1.03 times higher than the reduction in total aflatoxin at 200, 250, and 350 MPa, respectively. Meanwhile, there was 10 % increase in the concentration of $B_2$ between 200 and 250 MPa. Moreover, the concentration of $G_1$ increased by 4 % between 250 and 300 MPa, while concentration of $G_2$ rose by 6 and 7 % until 250 MPa and between 300 and 350 MPa, respectively (Figure 3.9 and Appendix Table A.9).

Finally, when the time was increased to 12 minutes at 200, 250, 300, and 350 MPa, the reductions in total aflatoxin were 33, 37, 39, and 49 %; and in aflatoxin $B_1$, 35, 37, 39, and 49 %, respectively. It was observed that the reduction in $B_1$ was the same as the total aflatoxin reduction at 250, 300, and 350 MPa; and 1.06 times higher than the reduction in total aflatoxin at 200 MPa. There was an increase of 6 % in the concentration of $B_2$ between 250 and 300 MPa. In addition, the concentration of $G_1$ increased at 200 MPa and between 300 and 350 MPa by 12 % and 13 %, respectively. Finally, pressurizing between 300 and 350 MPa caused the concentration of $G_2$ to increase by 6 % (Figure 3.10 and Appendix Table A.10).

The amounts of decrease in total aflatoxin and $B_1$ at each time interval were a little more at 30 °C than at 20 °C. This may indicate that the reduction rate was a direct function of temperature as well. The values are comparable to those in experiments with *Listeria, L. monocytogenes* and *L. innocua* in laboratory media, in which, modest increases in temperature (from 25 to 50°C) decreased decimal reduction time, D values from 50.8 to 22.4 min at 137.9 MPa and from 14.3 to 1.3 min at 344.7 MPa (Alpas et al., 1999). Again there were concentration increases in $B_2$, $G_1$, and $G_2$, which may be due to the conformational change of some of $B_1$ to $B_2$, $G_1$, and $G_2$.

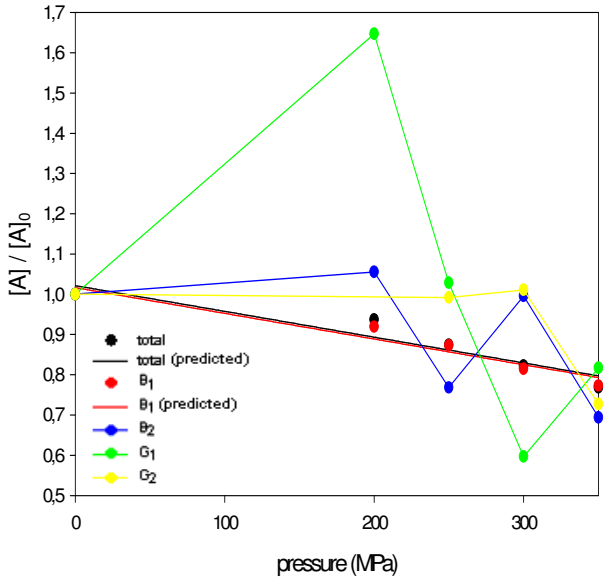


Figure 3.6 Changes in total aflatoxin ($R^2$ = 0.90), aflatoxin $B_1$ ($R^2$ = 0.94), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 4 min with various pressures at 30 °C

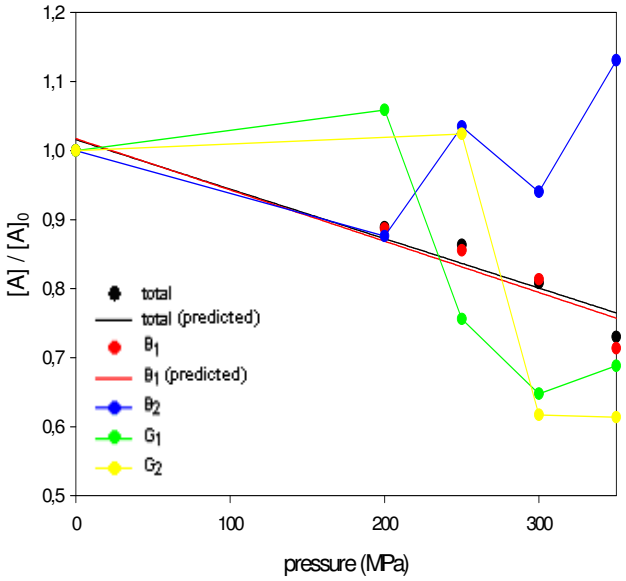


Figure 3.7 Changes in total aflatoxin ($R^2$ = 0.94), aflatoxin $B_1$ ($R^2$ = 0.92), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 6 min with various pressures at 30 °C

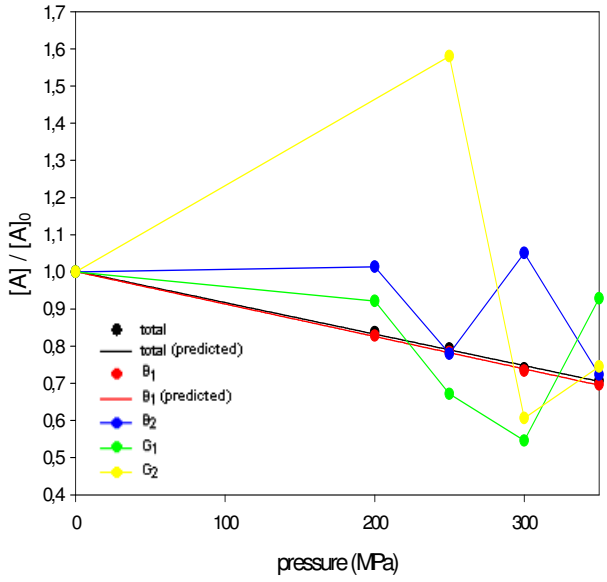


Figure 3.8 Changes in total aflatoxin ($R^2$ = 1), aflatoxin $B_1$ ($R^2$ = 1), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 8 min with various pressures at 30 ℃

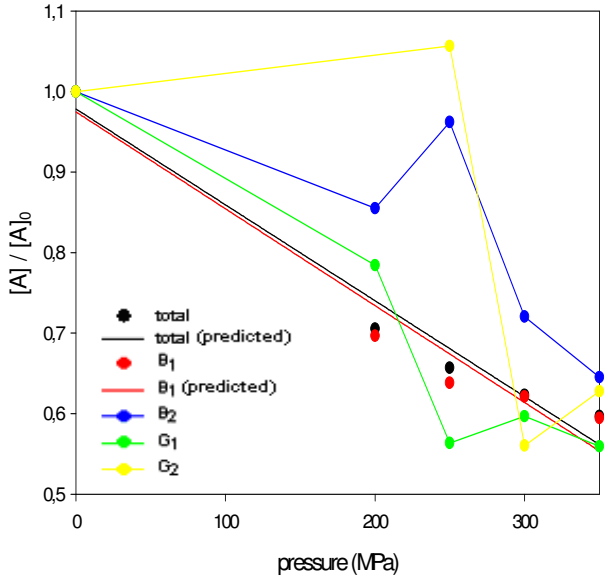


Figure 3.9 Changes in total aflatoxin ($R^2$ = 0.97), aflatoxin $B_1$ ($R^2$ = 0.95), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 10 min with various pressures at 30 ℃

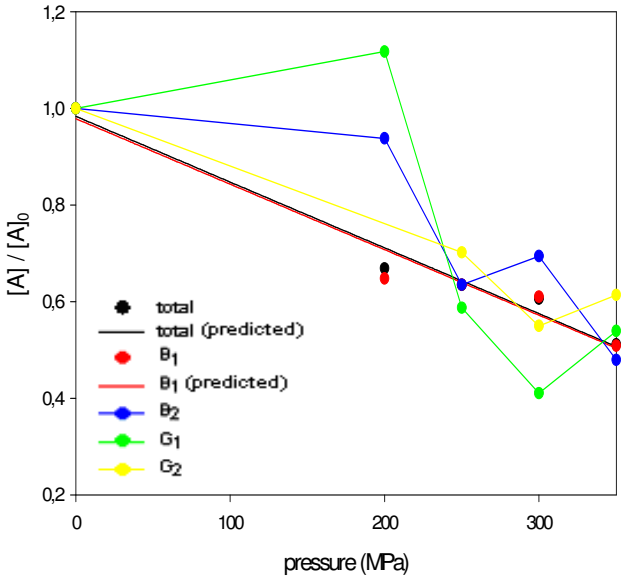


Figure 3.10 Changes in total aflatoxin ($R^2$ = 0.98), aflatoxin $B_1$ ($R^2$ = 0.96), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 12 min with various pressures at 30 °C

### 3.1.3. Effect of high hydrostatic pressure at 40 °C

From the inspection of the Figure 3.11 and Appendix Table A.11, for 4 minutes at 150, 200, 250, 300, and 350 MPa, the reductions in total aflatoxin were 3, 9, 15, 20, and 25 %; and in aflatoxin $B_1$, 3, 9, 15, 20, and 26 %, respectively. It was observed that the reduction in $B_1$ was the same as that in total aflatoxin at 150, 200, 250, and 300 MPa; and 1.04 times higher than the reduction in total aflatoxin at 350 MPa. The concentration of $B_2$ showed an increase of 7 and 5 % between 250 and 300 MPa and between 300 and 350 MPa, respectively, while $G_1$ increased by 14 and 8 % at 150 and between 150 and 200 MPa, respectively and $G_2$ increased by 9 % between 250 and 300 MPa.

According to Figure 3.12 and Appendix Table A.12, reductions caused at pressures of 150, 200, 250, 300, and 350 MPa (6 min) in total aflatoxin were about 9, 11, 15, 22, and 28 %; and in aflatoxin $B_1$, 9, 12, 15, 23, and 29 %, respectively. Accordingly, the reduction in $B_1$ was the same as total aflatoxin reduction at 150 and 250 MPa; and 1.09, 1.05, and 1.04 times higher than the reduction in total aflatoxin at 200, 300, and 350 MPa, respectively. There were 11 % and 54 % increases in the concentration of $B_2$ between 150 and 200 MPa and between 250 and 300 MPa,

respectively, while $G_1$ concentration showed a rise of 6, 8, and 29 % at 150, between 150 and 200 MPa and between 300 and 350 MPa, respectively. Finally, $G_2$ concentration rose by 40 % between 300 and 350 MPa, reaching its initial value.

Moreover, the percentages of reduction in total aflatoxin for 8 minutes at 150, 200, 250, 300 and 350 MPa were 16, 17, 23, 28, and 31 %; and in aflatoxin $B_1$, 17, 17, 24, 28, and 31 %, respectively. It was observed that the reduction in $B_1$ was the same as total aflatoxin reduction at 200, 300, and 350 MPa; and 1.06 and 1.04 times higher than the reduction in total aflatoxin at 150 and 250 MPa, respectively. The concentration of $B_2$ increased by 14 % during the application of pressure between 200 and 250 MPa. The concentration increase of $G_1$ was observed at 150 MPa as 16 %, while amount of $G_2$ rose by 39 % between 300 and 350 MPa (Figure 3.13 and Appendix Table A.13).

As Figure 3.14 and Appendix Table A.14 indicate, the percent reductions in total aflatoxin at high hydrostatic pressures of 150, 200, 250, 300, and 350 MPa for 10 minutes were about 27, 32, 36, 38, and 43 %; and in aflatoxin $B_1$, 27, 32, 37, 39, and 45 %, respectively. According to these results, the reductions in total aflatoxin and $B_1$ almost coincide, $B_1$ reduction being the same as total aflatoxin reduction at 150 and 200 MPa; and 1.03, 1.03, and 1.05 times higher than the total aflatoxin reduction at 250, 300, and 350 MPa, respectively. For $B_2$, there was a concentration increase of 20 % between 200 and 250 MPa. For $G_1$, there were two concentration increases (4 and 1 %) between 150 and 200 MPa and between 300 and 350 MPa, respectively. There was also a concentration increase (39 %) of $G_2$ which was observed between 300 and 350 MPa.

Finally, the percentages in reduction in total aflatoxin under pressures of 150, 200, 250, 300, and 350 MPa for 12 minutes were 29, 34, 37, 41, and 49 %; and in aflatoxin $B_1$; 30, 37, 37, 41, and 49 %, respectively. Accordingly, the reduction in aflatoxin $B_1$ was the same as total aflatoxin reduction at 250, 300, and 350 MPa; and 1.03 and 1.09 times higher than that of total aflatoxin under 150 and 200 MPa, respectively. There was again increase of concentration in $B_2$, which was 33 % between 150 and 200 MPa. While $G_1$ concentration showed a rise of 31 % between

150 and 200 MPa, $G_2$ concentration rose by 23 % between 300 and 350 MPa (Figure 3.15 and Appendix Table A.15).

At pressures of 100 and 200 MPa, decreases in microbial populations (Gram-positive and Gram-negative bacteria, moulds and yeasts, as well as spores of Gram-positive bacteria) in vegetables were not significant, whereas the populations of all the micro-organisms tested decreased considerably at a pressure of 300 MPa. A pressure of 300 MPa at 10°C for 20 min was required to completely reduce the population of *Saccharomyces cerevisiae*, and a pressure of 350 MPa was needed to reduce most of the Gram-negative bacteria and moulds (Arroyo et al., 1997). However, in the experiments, 150 and 200 MPa were found to be also effective on total aflatoxin and $B_1$, decreasing ca. 29-37 % of them.

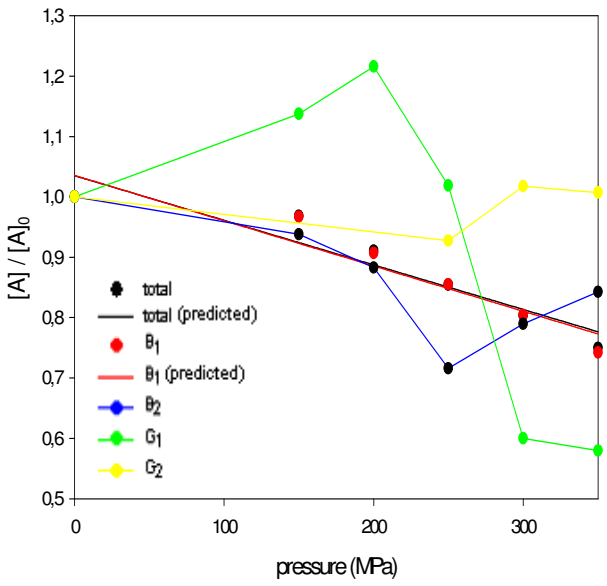


Figure 3.11 Changes in total aflatoxin ($R^2$ = 0.90), aflatoxin $B_1$ ($R^2$ = 0.90), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 4 min with various pressures at 40 °C

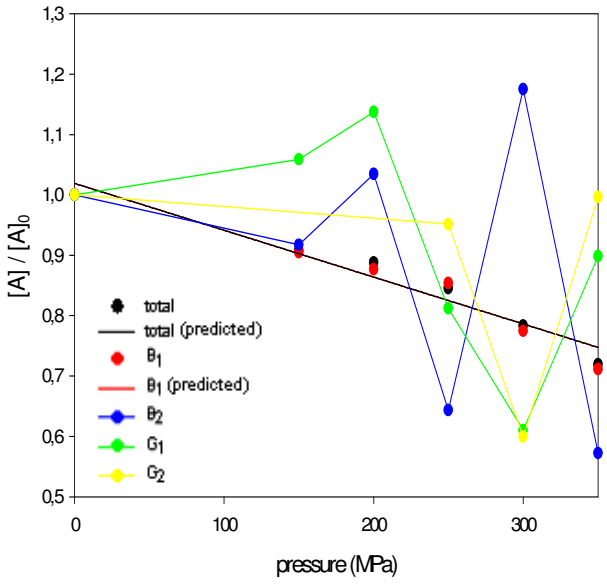


Figure 3.12 Changes in total aflatoxin ($R^2$ = 0.95), aflatoxin $B_1$ ($R^2$ = 0.95), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 6 min with various pressures at 40 °C

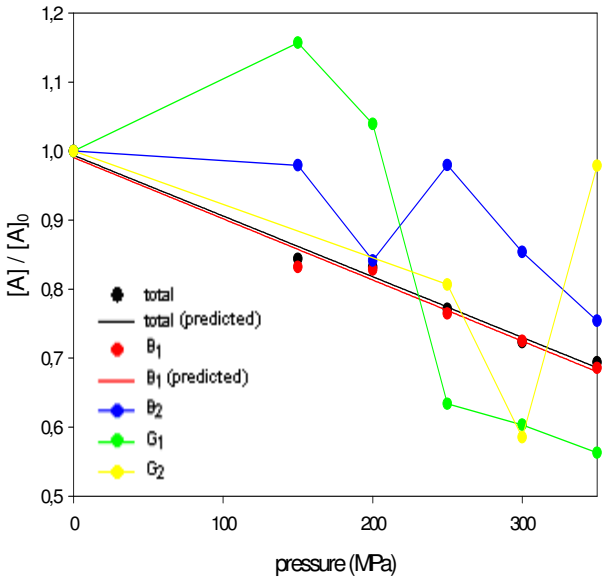


Figure 3.13 Changes in total aflatoxin ($R^2$ = 0.99), aflatoxin $B_1$ ($R^2$ = 0.98), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 8 min with various pressures at 40 °C

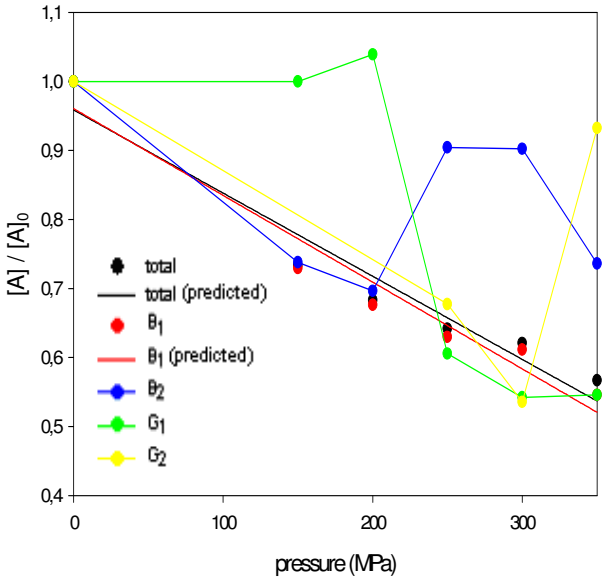


Figure 3.14 Changes in total aflatoxin ($R^2$ = 0.94), aflatoxin $B_1$ ($R^2$ = 0.95), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 10 min with various pressures at 40 ℃

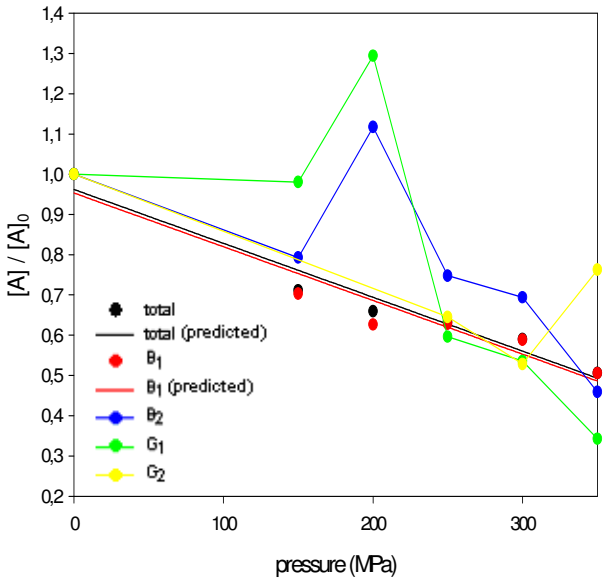


Figure 3.15 Changes in total aflatoxin ($R^2$ = 0.96), aflatoxin $B_1$ ($R^2$ = 0.93), $B_2$, $G_1$, and $G_2$ contents in red pepper treated for 12 min with various pressures at 40 ℃

As the results indicate, the greatest effect of high hydrostatic pressure, temperature and the time parameters was obtained at 350 MPa, 40 °C, and 12 minutes as 49 % decreases both in the concentrations of total aflatoxin and aflatoxin $B_1$ were observed. In conclusion, percent reductions in total aflatoxin and $B_1$ were found to be directly proportional to pressure, time and temperature. There is also another evidence of combined effect of pressure and temperature that high hydrostatic pressure, in combination with temperature, is a powerful tool affecting the activity of β-glucanase from barley malt (Buckow et al., 2005).

Since the percent reductions in total aflatoxin and $B_1$ were almost the same, it is unlikely that high hydrostatic pressure changed the conformation of $B_1$ to one of $B_2$, $G_1$, and $G_2$. However, this change may have occured to a small extent and there may also have been a conformational change of $B_2$, $G_1$ and $G_2$ from one to another. Moreover; because the initial amounts of $B_2$, $G_1$, and $G_2$ were very low compared to that of $B_1$ and total aflatoxin, the changes in their concentrations were not considerably altered.

An explanation of how aflatoxin $B_1$ was reduced in red pepper at high hydrostatic pressures (Figure 3.16) might be deduced from the ammoniation of aflatoxin $B_1$ in which the hydrolytic opening of the lactone ring is followed by decarboxylation (Cucullu et al., 1976). Similar explanations are made for the effect of high temperature application (Guerzoni, 1999) and fermentation (Nout 1994) on the structure of aflatoxin $B_1$, as well.

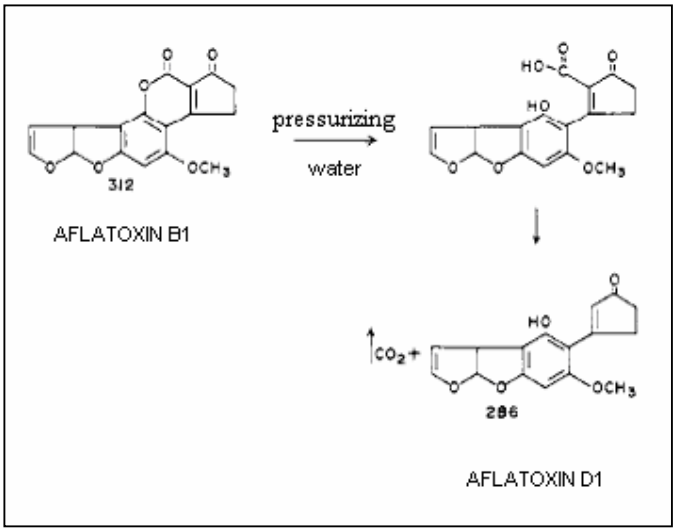


Figure 3.16 An expected scheme for the change in aflatoxin $B_1$ structure during the application of high hydrostatic pressure

In order to compare the amount of aflatoxin decrease in this study with other studies regarding aflatoxin elimination, several kinds of literature were examined. For instance; autoclaving at 120 $^oC$ and at 0.0105 $kg/mm^2$ is known to reduce toxins from 9,000 ppb to 350 ppb (96 %) , while gamma-irradiation at a level of 5 M rad would destroy 80 % of the aflatoxins in a sample (ikisan.com, 2000). In addition, reduction of aflatoxin (99.5 %) was achieved with grains processed at 250°C for 30 minutes (Oluwafemi, 2004). Moreover, roasting of peanut seeds at 150 $^oC$ for 30 min led to 70 % reductions in aflatoxin $B_1$ (Ogunsanwo et al., 2004). Compared to these results; 49 % reduction in aflatoxin $B_1$ indicates that in this study, higher pressure and temperature as well as long time period should be utilized in order to achieve greater reduction in aflatoxin content. The low reduction may be due to the low moisture content of red pepper, which as a result decreased the effect of the hydrostatic pressure.

### 3.2. Reaction Rates at Different Pressures

Since the destruction of aflatoxin is of great concern in the scientific area that deal with mycotoxins; the reaction which is of importance in this study is the one that causes a decrease in aflatoxin concentration. In this study, decreases in total and $B_1$ aflatoxin levels were observed, so in order to represent the overall effect of high

hydrostatic pressure on dried and powdered red pepper, the reaction which results in the smaller amount of **total** aflatoxin will be considered in the below sections.

According to the order determining methods (2.6.1), the order of the reaction at each pressure was supposed to be zero order due to the higher correlation coefficients of the plots of ([A] vs. t) than graphs (ln [A] vs. t), and (1/[A] vs. t) as shown in Appendix Table A.16. Thus, the reaction rate constant is equal to the negative value of the slope of plot ([A] vs. t) at each pressure-time combination. Additionally, here, zero-order reaction means that the reaction rate is independent of total aflatoxin concentration.

In order to determine the activation energies, (ln k vs. 1/T) graphs were plotted at each pressure. The slope of each graph gives $-E_a/R$ (Figure 2.7).

### 3.2.1. Reaction rate at 150 MPa

150 MPa was only tried at 40 °C because at 20 or 30 °C its effect on food possibly would be too low to be worth studying. When the samples were pressurized under 150 MPa at 40 °C; the overall reaction rate was found to be 0.8554 ppb/min (Figure 3.17 and Table 3.1).

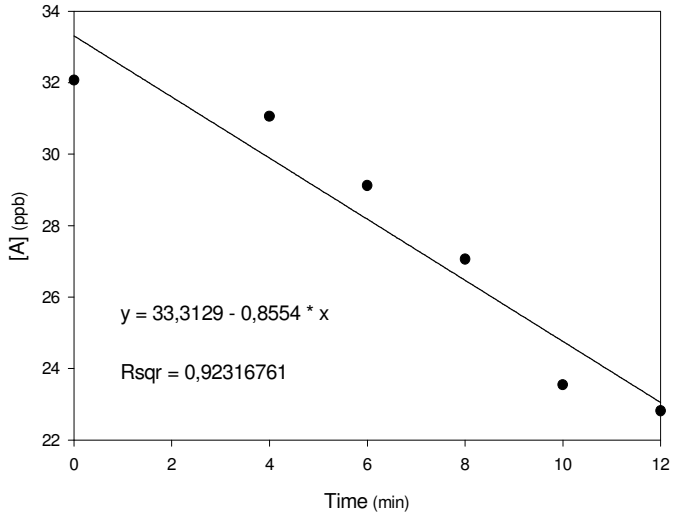


Figure 3.17 Change in total aflatoxin content with time at 40 °C and 150 MPa

Since there was a k value at only one temperature (40 °C) at 150 MPa, it was impossible to determine the activation energy at this pressure.

### 3.2.2. Reaction rates and activation energy at 200 MPa

The reaction at 200 MPa had a k value of 0.8964, 0.9361 and 0.9574 ppb/min at 20, 30 and 40°C, respectively (Figures 3.18, 3.19, and 3.20, and Table 3.1).

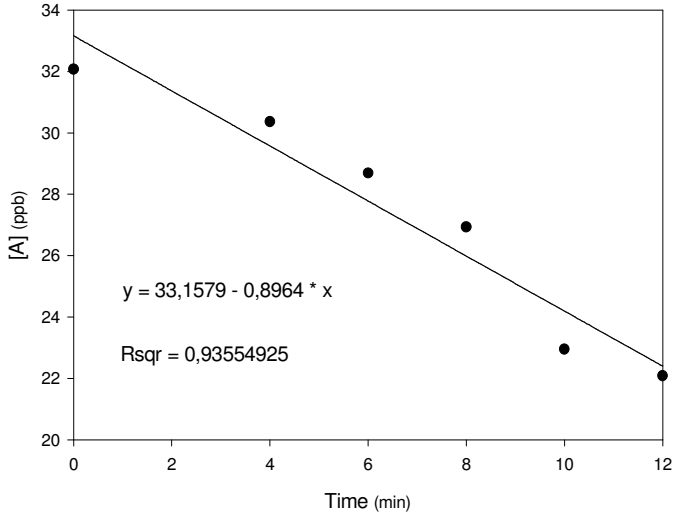


Figure 3.18 Change in total aflatoxin content with time at 20 °C and 200MPa

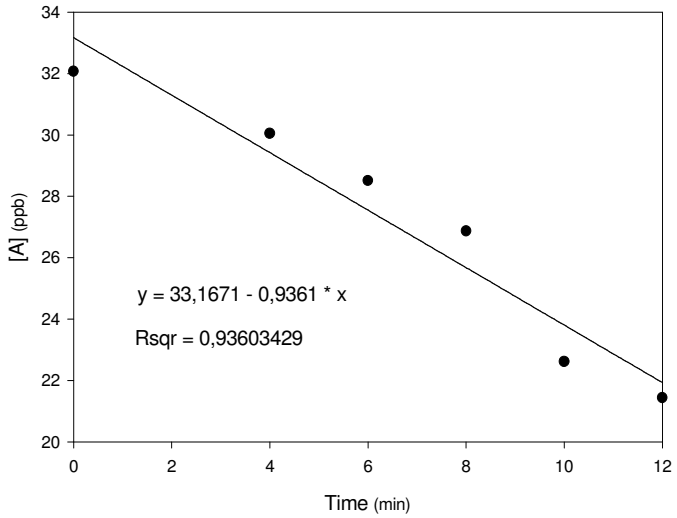


Figure 3.19 Change in total aflatoxin content with time at 30 °C and 200 MPa

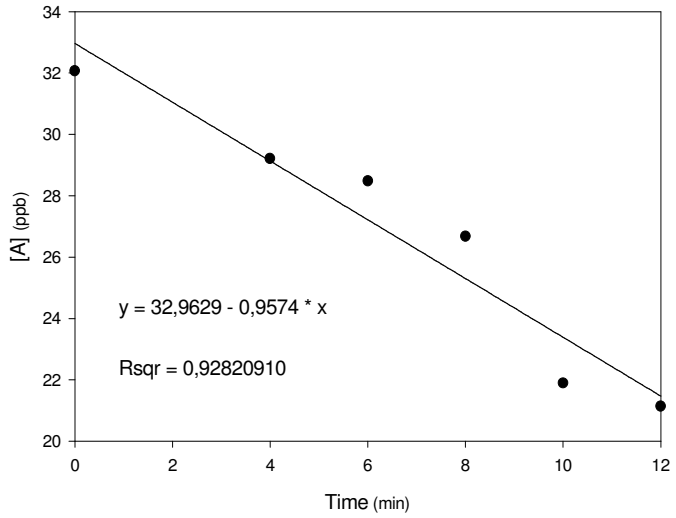


Figure 3.20 Change in total aflatoxin content with time at 40 °C and 200 MPa

Figures 3.18, 3.19, and 3.20 show clearly that, the reaction rate increases as the temperature was increased, which was the expected case. In addition, the reaction rate at 200 MPa and 20 °C was 1.05 times higher than the reaction rate at 150 MPa and 40 °C. Therefore, as the pressure was increased, the reaction rate also increased. In other words, the reaction proceeded faster at 200 MPa than at 150 MPa.

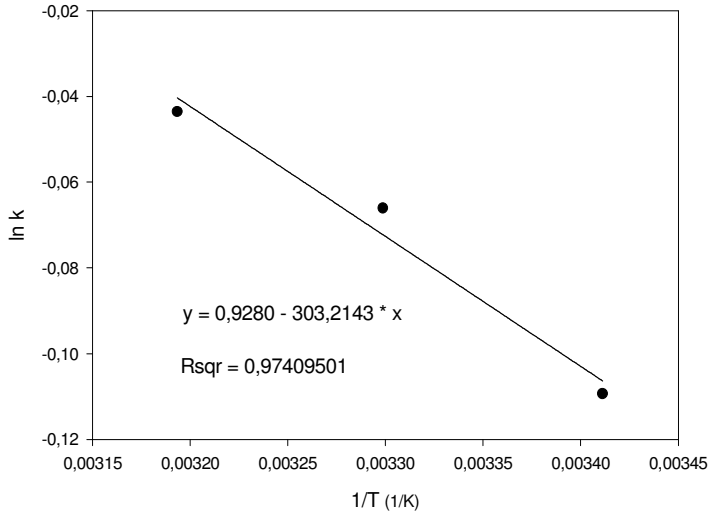


Figure 3.21 Arrhenius plot for evaluation of activation energy at 200 MPa

$$-\frac{E_a}{R} = -303.2143\,K \tag{3.1}$$

$$E_a = 303.2143\,K * 1.987\,\frac{cal}{gmol\,K} \tag{3.2}$$

$$E_a = 602.49\,\frac{cal}{gmol} \approx 0.602\,\frac{kcal}{gmol} \tag{3.3}$$

The activation energy found at 200 MPa is very low (Fig. 3.21). This shows that the reaction rates were not much affected by temperature change at 200 MPa. In other words, the reaction rates at 20, 30, and 40 °C at 200 MPa were not very different from each other. Additionally, the low activation energy may indicate that the intermediate complex (reactant-product) was formed very easily, without requiring a high amount of energy.

### 3.2.3. Reaction rates and activation energy at 250 MPa

At 250 MPa, the reaction rate constants were 2.3866, 2.4914, and 2.5124 ppb/min at 20, 30, and 40 °C, respectively (Figures 3.22, 3.23, and 3.24, and Table 3.1).

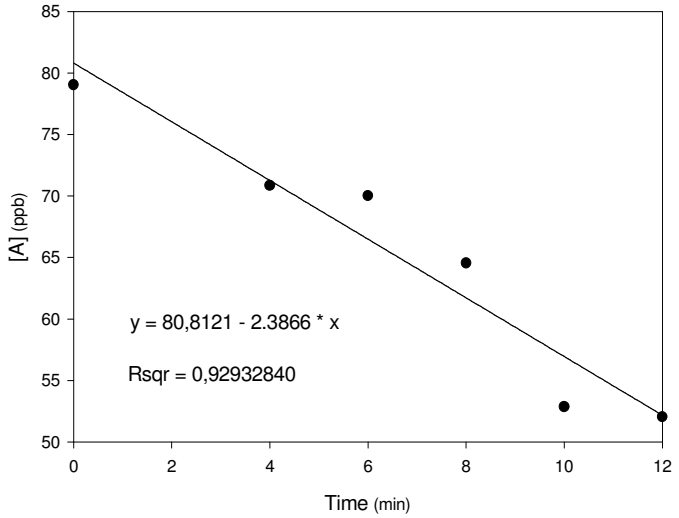


Figure 3.22 Change in total aflatoxin content with time at 20 °C and 250 MPa

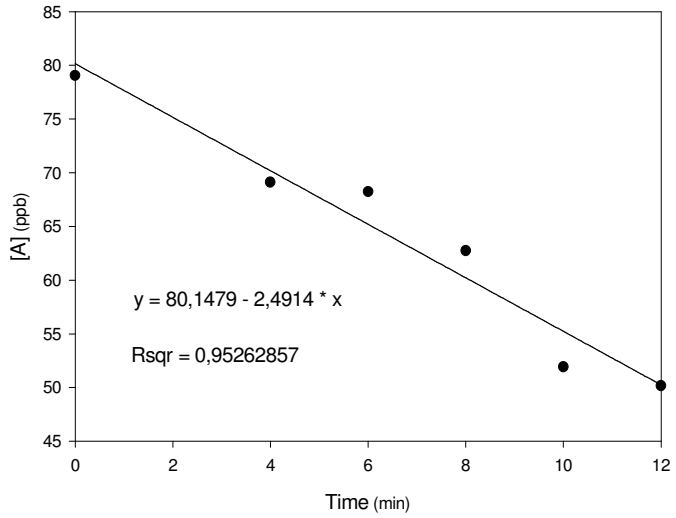


Figure 3.23 Change in total aflatoxin content with time at 30 °C and 250 MPa

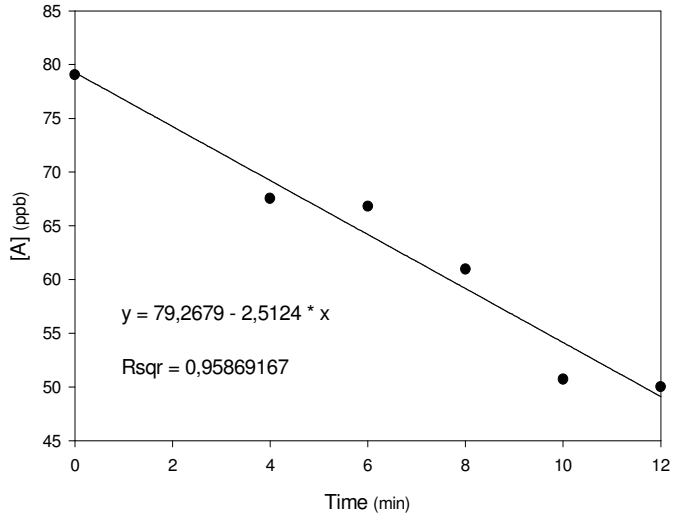


Figure 3.24 Change in total aflatoxin content with time at 40 °C and 250 MPa

Reaction rate constants increased as the temperature was increased at 250 MPa (Figure 3.22, 3.23, and 3.24). Moreover, the reaction rate at 250 MPa and 20 °C was 2.49 times higher than the reaction rate at 200 MPa and 40 °C. This clearly indicates that as the pressure was increased from 200 to 250 MPa, the reaction rate rose dramatically.

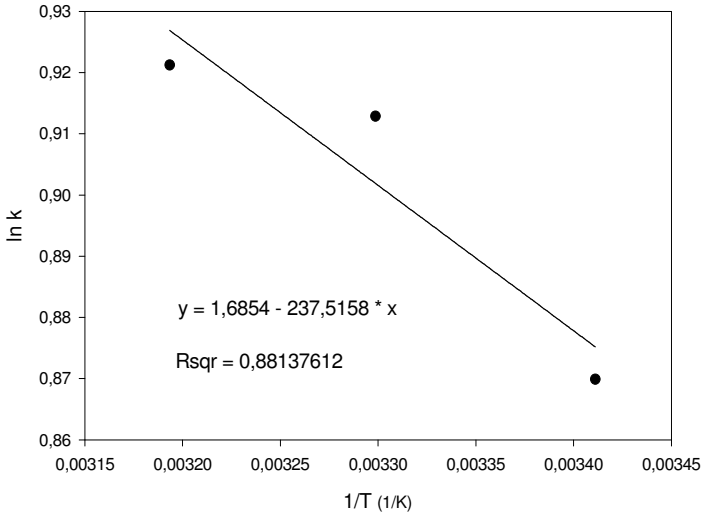


Figure 3.25 Arrhenius plot for evaluation of activation energy at 250 MPa

$$-\frac{E_a}{R} = -237.5158\,K \tag{3.4}$$

$$E_a = 237.5158\,K * 1.987\,\frac{cal}{gmol\,K} \tag{3.5}$$

$$E_a = 471.9\,\frac{cal}{gmol} \approx 0.472\,\frac{kcal}{gmol} \tag{3.6}$$

As the pressure was increased from 200 MPa to 250 MPa, the activation energy decreased (Fig. 3.25), which means that a less activation energy was required for the reaction at 250 MPa compared to 200 MPa. The activation energy ($E_a$) at 200 MPa was 1.28 times higher than at 250 MPa. Additionally, the reaction rates became less temperature dependent as the pressure was increased from 200 MPa to 250 MPa.

### 3.2.4. Reaction rates and activation energy at 300 MPa

Under pressure of 300 MPa, the relating rate constants at 20, 30, and 40 °C were found to be 3.0393, 3.1069, and 3.1551 ppb/min, respectively (Figures 3.26, 3.27, and 3.28, and given in Table 3.1)

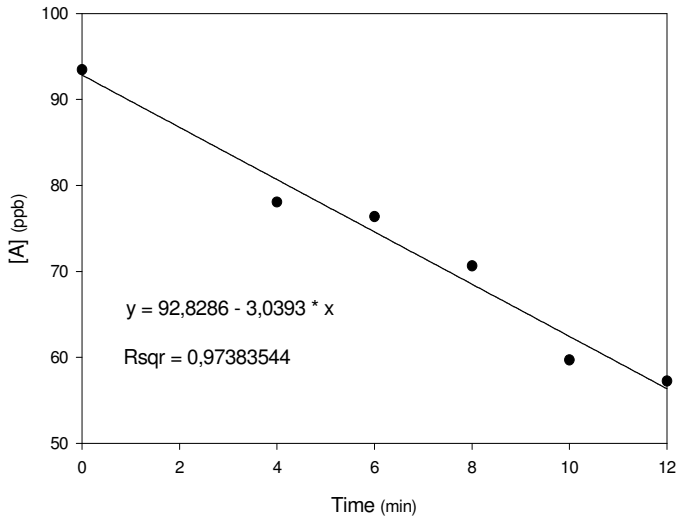


Figure 3.26 Change in total aflatoxin content with time at 20 °C and 300 MPa

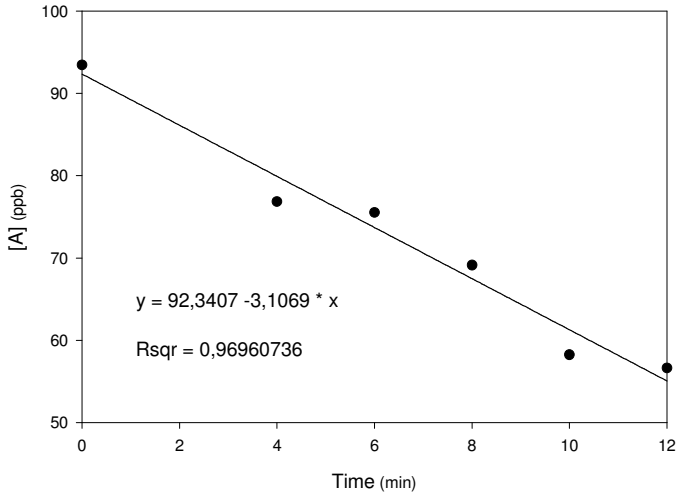


Figure 3.27 Change in total aflatoxin content with time at 30 °C and 300 MPa

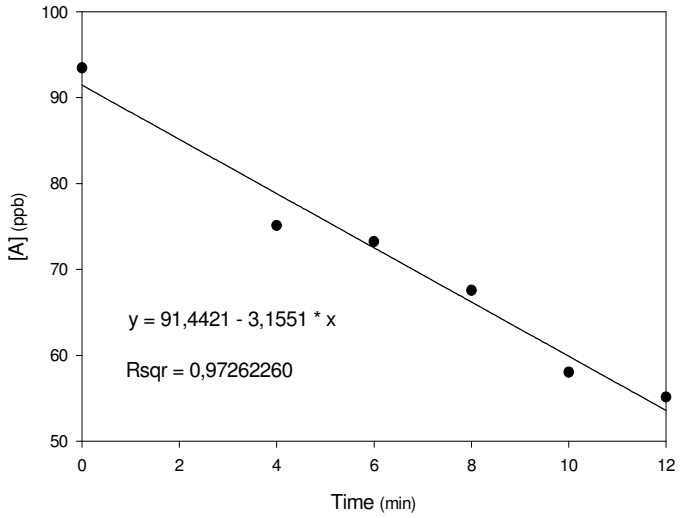


Figure 3.28 Change in total aflatoxin content with time at 40 °C and 300 MPa

Just like in (3.2.2.) and (3.2.3.); the reaction rate was directly proportional to the temperature at 300 MPa. The reaction rate at 300 MPa and 20 °C was 1.21 times higher than the reaction rate at 250 MPa and 40 °C. Again, this means that the reaction rate was directly proportional to the pressure as well.

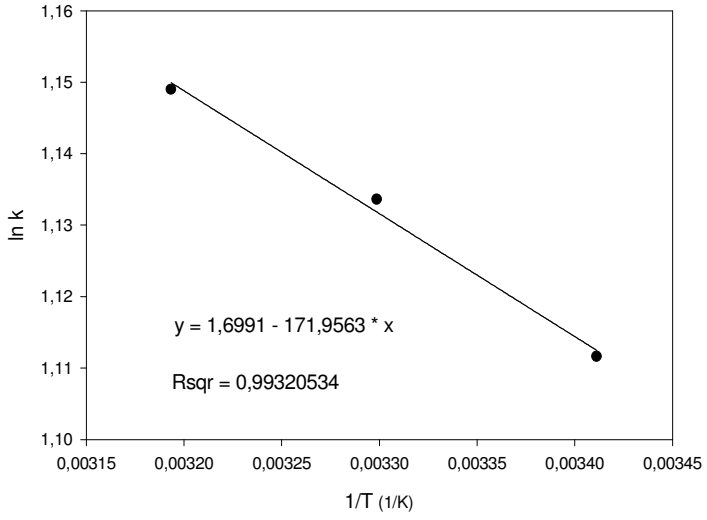


Figure 3.29 Arrhenius plot for evaluation of activation energy at 300 MPa

$$-\frac{E_a}{R} = -171.9563\,K \tag{3.7}$$

$$E_a = 171.9563\,K * 1.987 \frac{cal}{gmol\,K} \tag{3.8}$$

$$E_a = 341.7\ \frac{cal}{gmol} \approx 0.342 \frac{kcal}{gmol} \tag{3.9}$$

The activation energy at 250 MPa (0.472 kcal/mol) was 1.38 times higher than that at 300 MPa (0.342 kcal/mol). Again, the energy required for the formation of the reaction was lower at 300 MPa (Figure 3.29), compared to 250 and 200 MPa. Moreover, it can be concluded that temperature dependence of the reaction rate decreased as the pressure was increased.

### 3.2.5. Reaction rate and activation energy at 350 MPa

At 350 MPa, the reaction rate constants were 3.5185, 3.5507, and 3.6387 ppb/min at 20, 30, and 40 °C, respectively, as are illustrated in Figures 3.30, 3.31, and 3.32 and Table 3.1.

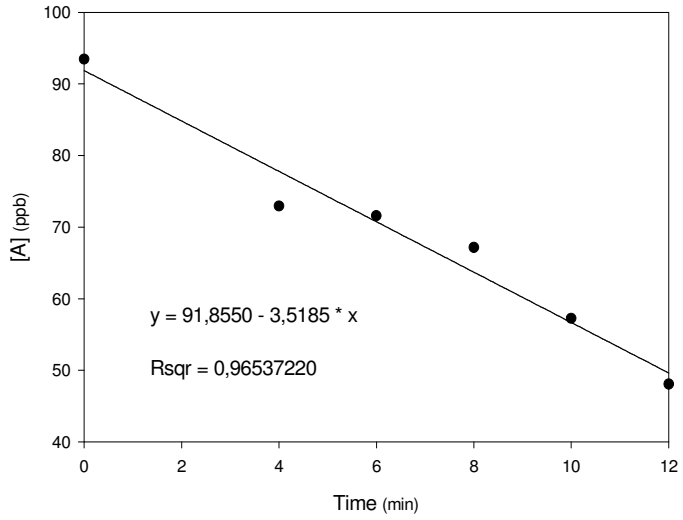


Figure 3.30 Change in total aflatoxin content with time at 20 °C and 350 MPa

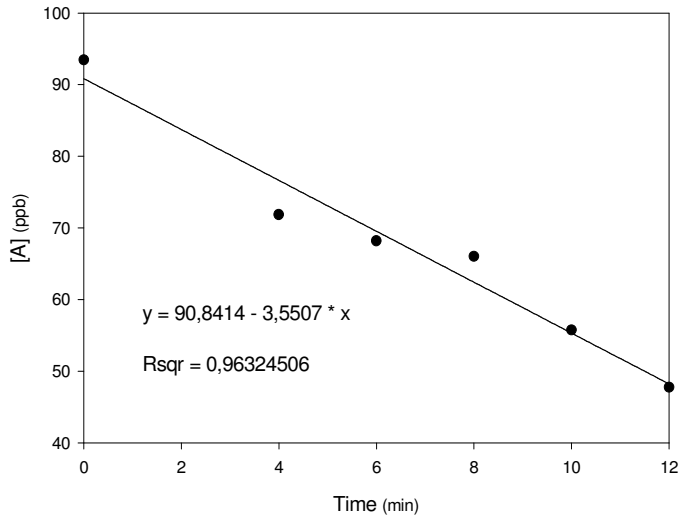


Figure 3.31 Change in total aflatoxin content with time at 30 °C and 350 MPa

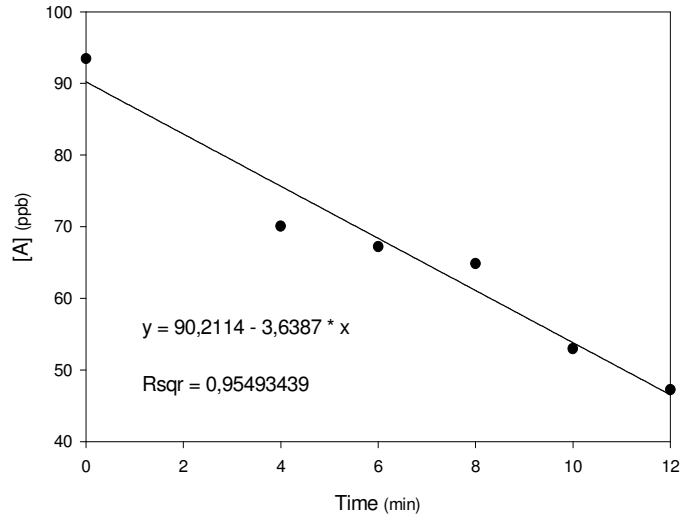


Figure 3.32 Change in total aflatoxin content with time at 40 °C and 350 MPa

As demostrated in Figures 3.30, 3.31, and 3.32; at 350 MPa, as the temperature was increased, the reaction rate also increased. The reaction rate at 350 MPa and 20 °C was 1.12 times higher than that at 300 MPa and 40 °C. As a result, the reaction rate was directly proportional to the pressure.

Table 3.1 k values at different pressures and temperatures

| k (ppb/min) | T (°C) | P (MPa) |
|---|---|---|
| 0.8554 | 40 | 150 |
| 0.8964 | 20 | 200 |
| 0.9361 | 30 | |
| 0.9574 | 40 | |
| 2.3866 | 20 | 250 |
| 2.4914 | 30 | |
| 2.5124 | 40 | |
| 3.0393 | 20 | 300 |
| 3.1069 | 30 | |
| 3.1551 | 40 | |
| 3.5185 | 20 | 350 |
| 3.5507 | 30 | |
| 3.6387 | 40 | |

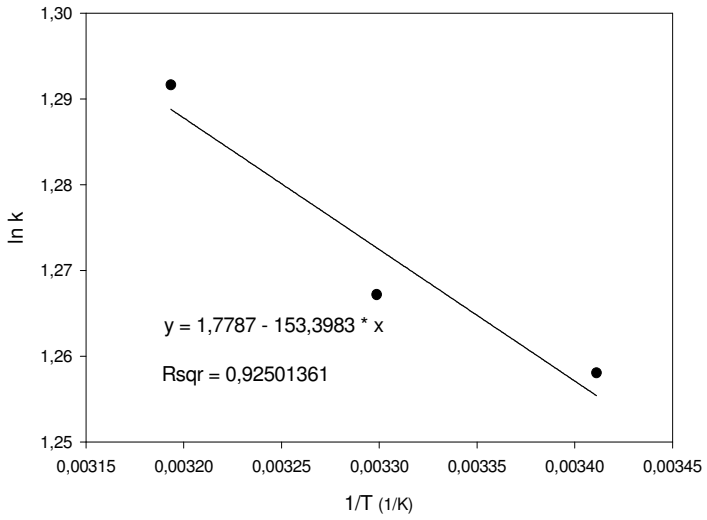


Figure 3.33 Arrhenius plot for evaluation of activation energy at 350 MPa

$$-\frac{E_a}{R} = -153.3983\,K \tag{3.10}$$

$$E_a = 153.3983\,K * 1.987\,\frac{cal}{gmol\,K} \tag{3.11}$$

$$E_a = 304.8 \frac{cal}{gmol} \approx 0.305 \frac{kcal}{gmol} \quad (3.12)$$

The activation energy ($E_a$) at 300 MPa is 1.12 times higher than that at 350 MPa. As it can be seen from Figures 3.21, 3.25, 3.29, and 3.33 and Table 3.2, the activation energy decreased as the pressure was increased. This leads to two conclusions: First is that the energy required to form the reactant-product complex for the initiation of the reaction decreased as pressure was increased, therefore the reaction took place more easily at higher pressures. Second is that, the reaction rates became less temperature dependent as the pressure was increased. In other words, temperature was less effective on reaction rates at higher pressures.

Table 3.2 Activation energies at different pressures

| Pressure (MPa) | Activation Energy (kcal/mol) |
|---|---|
| 200 | 0.602 |
| 250 | 0.472 |
| 300 | 0.342 |
| 350 | 0.305 |

The value of $E_a$ is in the range 210 to about 418 kJ/gmol (50-100 kcal/gmol) for vegetative cells and spores and much less for enzymes and vitamins (Jackson & Lamb, 1981). Compared to the destruction of vegetative cells and spores, activation energy required for the destruction of aflatoxin in red pepper under high hydrostatic pressure within the range of 150-350 MPa is very low. For the vitamin losses the activation energy ranges between 20 and 30 kcal/mole and for the enzyme reactions between 10 and 30 kcal/mol (Sewald and DeVries, 2003). As a result, the activation energy of enzymatic reactions is approximately 17 to 98 times higher compared to the activation energy required for total aflatoxin degradation.

# CHAPTER 4

# CONCLUSIONS

According to the experimental results obtained in this study, the conclusions can be listed as follows:

1. The most effective pressure, time, and temperature combination for the percent reduction of total aflatoxin and $B_1$ was 350 MPa, 12 minutes, and 40 ºC, which caused 49 % reduction in both total aflatoxin and $B_1$.
2. In each hydrostatic pressure treatment, there were both increases and decreases in the concentrations of $B_2$, $G_1$, and $G_2$ types with time.
3. The percent reductions of total aflatoxin and $B_1$ were found to be directly proportional to pressure, time, and temperature.
4. Reduction rate of total aflatoxin was directly proportional to pressure and temperature.
5. The activation energy of the reaction, which led to the reduction in total aflatoxin, decreased as the hydrostatic pressure was increased. Therefore, at higher pressures, the effect of temperature on reaction rate was smaller. Moreover, the reaction proceeded more easily at higher pressures.

## RECOMMENDATIONS

High pressure seems to be effective on aflatoxin in dried and powdered red pepper, decreasing total aflatoxin and $B_1$ level. However, what biological and chemical changes that high pressure causes on aflatoxin needs to be explored.

350 MPa may be very low for aflatoxin destruction, since molds are resistant to almost 250 MPa. For instance, the minimum pressure for inactivation of conidiospores of *Aspergillus fumigatus* is 300 MPa (Eicher et al., 1998). Therefore pressures above 350 MPa should be applied on different samples.

Moreover, under economical conditions, higher temperatures and longer time periods should be utilized because pressure is almost not effective at room temperatures (Tauscher, 1999) and if time period is increased, better results could be obtained.

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Table A.1 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 4 min at 20 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 30.36 | 28.28 | 1.31 | 0.77 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 70,86 | 65.11 | 2.67 | 2.41 | 0.67 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 78.05 | 69.17 | 3.73 | 3.56 | 1.59 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 72.92 | 64.36 | 4.15 | 3.14 | 1.27 | |

Table A.2 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 6 min at 20 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 28.69 | 26.86 | 1.26 | 0.57 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 70.03 | 64.29 | 2.56 | 2.16 | 1.02 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 76.37 | 67.45 | 3.55 | 1.94 | 3.43 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 71.57 | 62.51 | 5.11 | 2.31 | 1.64 | |

Table A.3 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 8 min at 20 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 26.93 | 25.20 | 1.10 | 0.63 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 64.55 | 58.84 | 3.38 | 1.55 | 0.78 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 70.63 | 63.00 | 3.25 | 2.67 | 1.71 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 67.15 | 59.94 | 3.60 | 1.59 | 2.02 | |

Table A.4 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 10 min at 20 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 22.95 | 21.42 | 1.14 | 0.39 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 52.87 | 47.93 | 2.65 | 1.53 | 0.76 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 59.69 | 52.74 | 2.43 | 2.85 | 1.67 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 57.24 | 49.88 | 2.94 | 1.91 | 2.51 | |

Table A.5 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 12 min at 20 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 22.09 | 20.70 | 0.92 | 0.47 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 52.05 | 46.56 | 3.22 | 1.55 | 0.72 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 57.22 | 50.92 | 2.39 | 2.37 | 1.54 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 48.07 | 42.28 | 2.28 | 1.50 | 2.01 | |

Table A.6 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 4 min at 30 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 30.05 | 27.68 | 1.53 | 0.84 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 69.12 | 63.05 | 2.65 | 2.19 | 1.23 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 76.84 | 67.74 | 4.49 | 1.76 | 2.85 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 71.84 | 64.25 | 3.13 | 2.41 | 2.05 | |

Table A.7 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 6 min at 30 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 28.51 | 26.70 | 1.27 | 0.54 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 68.23 | 61.78 | 3.57 | 1.61 | 1.27 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 75.52 | 67.63 | 4.24 | 1.91 | 1.74 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 68.20 | 59.34 | 5.10 | 2.03 | 1.73 | |

Table A.8 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 8 min at 30 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 26.87 | 24.93 | 1.47 | 0.47 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 62.74 | 56.66 | 2.69 | 1.43 | 1.96 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 69.12 | 61.06 | 4.74 | 1.61 | 1.71 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 66.03 | 57.93 | 3.26 | 2.74 | 2.10 | |

Table A.9 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 10 min at 30 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 22.62 | 20.98 | 1.24 | 0.40 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 51.93 | 46.10 | 3.32 | 1.20 | 1.31 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 58.24 | 51.65 | 3.25 | 1.76 | 1.58 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 55.76 | 49.43 | 2.91 | 1.65 | 1.77 | |

Table A.10 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 12 min at 30 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 21.44 | 19.51 | 1.36 | 0.57 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 50.16 | 45.85 | 2.19 | 1.25 | 0.87 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 56.61 | 50.72 | 3.13 | 1.21 | 1.55 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 47.75 | 42.27 | 2.16 | 1.59 | 1.73 | |

Table A.11 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 4 min at 40 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 150 |
| | After pressurizing | 31.06 | 29.12 | 1.36 | 0.58 | - | |
| | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 29.21 | 27.31 | 1.28 | 0.62 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 67.54 | 61.75 | 2.47 | 2.17 | 1.15 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 75.09 | 66.89 | 3.56 | 1.77 | 2.87 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 70.06 | 61.71 | 3.80 | 1.71 | 2.84 | |

Table A.12 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 6 min at 40 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 150 |
| | After pressurizing | 29.12 | 27.25 | 1.33 | 0.54 | - | |
| | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 28.48 | 26.40 | 1.50 | 0.58 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 66.81 | 61.68 | 2.22 | 1.73 | 1.18 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 73.21 | 64.42 | 5.30 | 1.80 | 1.69 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 67.19 | 59.15 | 2.58 | 2.65 | 2.81 | |

Table A.13 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 8 min at 40 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 150 |
| | After pressurizing | 27.06 | 25.05 | 1.42 | 0.59 | - | |
| | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 26.68 | 24.93 | 1.22 | 0.53 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 60.96 | 55.23 | 3.38 | 1.35 | 1.00 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 67.56 | 60.28 | 3.85 | 1.78 | 1.65 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 64.83 | 57.01 | 3.40 | 1.66 | 2.76 | |

Table A.14 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 10 min at 40 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 150 |
| | After pressurizing | 23.54 | 21.96 | 1.07 | 0.51 | - | |
| | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 21.90 | 20.36 | 1.01 | 0.53 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 50.73 | 45.48 | 3.12 | 1.29 | 0.84 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 58.02 | 50.84 | 4.07 | 1.60 | 1.51 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 52.95 | 45.39 | 3.32 | 1.61 | 2.63 | |

Table A.15 Concentrations of total aflatoxin, aflatoxin $B_1$, $B_2$, $G_1$ and $G_2$ pressurized for 12 min at 40 °C at various pressures

| | | Aflatoxin | | | | | Pressure (MPa) |
|---|---|---|---|---|---|---|---|
| | | total | $B_1$ | $B_2$ | $G_1$ | $G_2$ | |
| Concentration (ppb) | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 150 |
| | After pressurizing | 22.81 | 21.16 | 1.15 | 0.50 | - | |
| | Initial | 32.07 | 30.11 | 1.45 | 0.51 | - | 200 |
| | After pressurizing | 21.14 | 18.86 | 1.62 | 0.66 | - | |
| | Initial | 79.05 | 72.23 | 3.45 | 2.13 | 1.24 | 250 |
| | After pressurizing | 50.02 | 45.37 | 2.58 | 1.27 | 0.80 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 300 |
| | After pressurizing | 55.13 | 48.93 | 3.13 | 1.58 | 1.49 | |
| | Initial | 93.44 | 83.16 | 4.51 | 2.95 | 2.82 | 350 |
| | After pressurizing | 47.25 | 42.02 | 2.07 | 1.01 | 2.15 | |

Table A.16 Correlation coefficient data for the reaction order determination at different pressures and temperature combinations

| Pressure (MPa) | Temp. (°C) | Correlation Coefficient ($R^2$) | | |
|---|---|---|---|---|
| | | Zero-order | First-order | Second-order |
| | | [A] vs. t | ln [A] vs. t | 1/[A] vs. t |
| 150 | 40 | **0.9232** | 0.9073 | 0.8895 |
| 200 | 20 | **0.9355** | 0.9149 | 0.8920 |
| | 30 | **0.9360** | 0.9123 | 0.8861 |
| | 40 | **0.9282** | 0.9027 | 0.8747 |
| 250 | 20 | **0.9293** | 0.9049 | 0.8773 |
| | 30 | **0.9526** | 0.9315 | 0.9046 |
| | 40 | **0.9587** | 0.9430 | 0.9194 |
| 300 | 20 | **0.9738** | 0.9656 | 0.9463 |
| | 30 | **0.9696** | 0.9648 | 0.9478 |
| | 40 | 0.9726 | **0.9773** | 0.9670 |
| 350 | 20 | **0.9654** | 0.9518 | 0.9121 |
| | 30 | **0.9632** | 0.9625 | 0.9335 |
| | 40 | 0.9549 | **0.9621** | 0.9420 |

## APPENDIX B

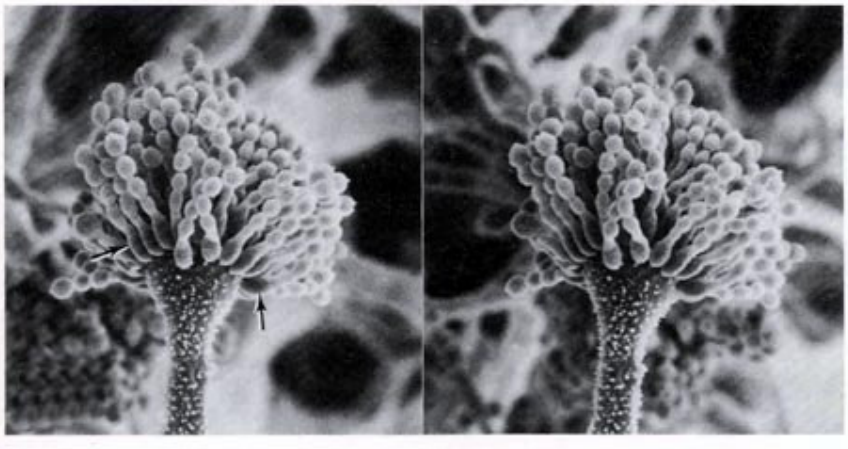

Figure B.1 Terminal portion of a conidiophore of A. flavus showing the basal portion of the vesicle and distribution of radiation phialides (arrows) X 1000 (Reddy and Farid, 2000)

Figure B.2 Phialides and chains of conidia of A. parasiticus illustrating basipetal development of conidia. Those at the base of the chains (arrows) are least mature X 3000 (Reddy and Farid, 2000)

Figure B.3 Fresh red pepper (left) & dried and powdered red pepper (right)

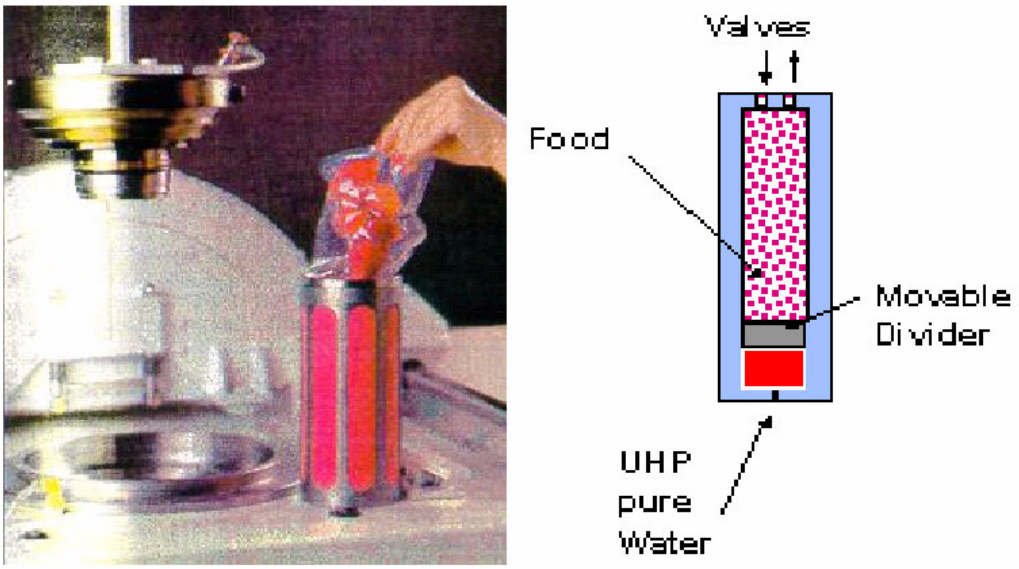


Figure B.4 Principle of isostatic pressing (Alpas, 2005)

Figure B.5 Hydrostatic pressure unit, Food Engineering Department, METU, Ankara

Figure B.6 HPLC unit (hp-Agilent 1100)