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Effect of some different technological treatments on the aflatoxins reduction in soybean seeds

Mohamed S. Shahat[1], Ahmed I. Hegaziy[1], Sherif Ramzy[2] and Mohamed Abdel Samie[1]

[1]*Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*
[2]*Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Giza, Egypt.*

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ABSTRACT

This study aimed to evaluate the effect of using some different technological treatments (such as fermentation, germination, blanching and roasting) on remove or degrade of the aflatoxins (AFs) in contaminated soybean seeds. The results revealed that all technological treatments carried out on contaminated soybean seeds exhibited variable reductions in aflatoxin B_1 (from 10.5 to 43.2%) and complete removal (100%) for aflatoxins G_1 and G_2. While, aflatoxin B_2 was not detected in the seeds after procedure the tested technological treatments. It can be concluded that the fermentation, germination, blanching and roasting treatments are not efficient to remove or degrade AFs from the tested seeds to the acceptable and recognized level (10 µg/kg) as reported by WHO (2014), where total reduction rates ranged from 13.0 to 44.8%. The roasting treatment showed the highest positive effect in reducing of all aflatoxins (from 51.63 to 28.50 µg/kg) with 44.8% reduction.

Key words: soybean seeds; aflatoxin B1; technological treatments.

Introduction

Soybean is an Asiatic leguminous plant cultivated in many parts of the world for its oil and proteins, which are extensively used in the manufacture of animal and human foodstuffs (Hepperly, 1985 and FAO, 2004). In Egypt, soybean acreages had declined drastically during the last twenty years, from about 42 thousand ha in 1991 to about 7.2 thousand ha in 2009 due to several biotic and abiotic factors negatively affecting soybean production. During 2008, Egypt imported 1,192,400 tons of soybean seed and 228,865 tons of soybean oil costing to $ 450 and $ 446 million, respectively. Climatic changes greatly affect soybean yield and yield components (Biabani *et al.,* 2008). Soybean is often attacked by fungal infections during cultivation, or post-harvest (in transit or in storage), significantly affecting its productivity. The seeds and infected harvest debris are the main sources of primary infections, and the level of seed damage depends on environmental conditions such as high relative humidity, dew, and temperatures above 25 ºC. The production reached 47.5 million tons during the 2006/2007 harvest season ranking (SAGPYA, 2010).

Soybean is the second important crop for Egyptian oil industries in the present time especially in new industrial areas and the private sector factories (6 October, Obour and 10 Ramadan cities). Soybean may use directly as food materials (soy flour or soy souse), it has many health benefits such as preventing of arteriosclerosis and blood pressure (El Agroudy *et al.,* 2011). Solo soybeans deem as a good source for many essential vitamins for human and animal's body system.

In Egypt, soybean began uses in the mid of seventies of the last century. Soybean is a highly source of protein in animal feed especially for chicken. In the last years and according to previous studies, soybean had shortage in cultivated areas in Egypt (El Agroudy *et al.,* 2011). this may serve the turned to soybean imported either for human food consumption, feed usage and oil production process, this lead urgently a study on safety of imported soybean and stored condition in Egypt.

Aflatoxins are complex compounds that excretes as secondary metabolites by toxigenic fungi mainly Aspergillus sp. (Sabry *et al.,* 2016). The during its growth on food and feed materials, recorded tissue damages in the presence of AFB1 in feeding meal, as a results of aflatoxin exposure it lead to case of disease called Aflatoxicosis (Aletor, 1990). Zambelli *et al.,* (2013) reported that

Corresponding Author: Hegazy A. I., Department of Food Science and Technology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. E-mail: ahmedhegazyh@yahoo.com

aflatoxin was detected in many maladies cases of cancer. It is lead to death in many countries such as western Indian outbreak on 1974, Kenyan case on 2004 which called Kenya episode. In Kenya one hundred twenty five person dead as they eat contaminated maize with aflatoxin (Reddy and Raghavender, 2007). Therefore, the purpose of the present study was to evaluate the effect of using some different technological treatments (such as fermentation, germination, blanching and roasting) on the degradation and reduction of aflatoxins in contaminated soybean seeds.

Materials and Methods

Materials:

Soybean samples:

A total samples of (20 kg) soybean seeds (*Glycine max L. Merr.*) were randomly collected from local markets at season of 2015. One kilogram of each sample was collected and stored in polyethylene bags at -18 ^{O}C until aflatoxins determination.

Chemicals:

Aflatoxins B_1, B_2, G_1 and G_2 standards were produced by Sigma Chemical Co. (St. Louis, Mo. USA). The precautions of aflatoxin handling were followed according to the brochure of producer. Other chemical used in the study (as chloroform, n-hexan, methanol and sodium chloride) were produced by El. Naser chemical company, Giza, Egypt.

Methods:

Experimental Treatments:

Preparation of aflatoxin stock solutions:

Stock standard solutions from each aflatoxin type (B_1, B_2, G_1 and G_2) were used. The solutions were prepared in toxins and pollutants food lab, National Research Center, Giza, Egypt, by dissolving 0.5 µg /ml. The standard solutions of aflatoxins B_1, B_2, G_1 and G_2 were stored at 4 C° and could be stable more than one year. The stock solution of aflatoxin was used as standard solution for determination by HPLC.

Inoculation of aflatoxins in soybean seeds:

The soybean seeds were cleaned and then artificially contaminated with aflatoxin (B_1, B_2, G_1 or G_2 dissolved in corn oil at concentration of 50µg/kg) to study the effect of tested technological treatments. Thereafter the contaminated seeds well homogenized and dried at 50^{o} C.

Technological treatments for contaminated soybean seeds:

1. Fermentation treatment: Fermentation of soybean seeds using lactic acid bacteria for 3 days at 37° C according to Abdellah *et al.* (2005). After that, the soybean seeds were dried at 50° C for 30 min.
2. Germination treatment: Germination of soybean seeds was continued for 3 days at 37° C according to Agrahar and Jha (2009). Then, soybean seeds were dried at 50° C for 30 min.
3. Blanching treatment: Blanching of soybean seeds at 100° C for 30 min according to Papa *et al.* (2012). Then, the soybean seeds were dried at 50 °C for 30 min.
4. Roasting treatment: Roasting of soybean seeds were carried out at 150° C for 30 min according to Papa *et al.* (2012).

Extraction of aflatoxins:

As according to AOAC (2007), twenty-five grams of each homogenized sample was taken and transferred to 500 ml glass-stoppered Erlenmeyer flask and 125 ml of methanol: water (55:45 V/V), 100 ml hexane and 2 gm sodium chloride were added. Shaking vigorously for 30 min on an orbital shaker then the mixture was filtered through Whitman No.1 filter paper and the filtrate was allowed to stand undisturbed when separation will occur within 30 minutes. 25 ml of lower aqueous methanol phase was taken in a separating funnel and 10 ml chloroform was added then shacked for 30-60 sec. Repeat extraction several times with chloroform for complete separation. The separating funnel was allowed to stand for some time until two layers formed then the lower chloroform layer was drained over anhydrous sodium sulfate into a 250 ml beaker. Collect in a beaker and evaporate combined chloroform extract in a water bath (50-60°C) near dryness and the residue was washed twice with chloroform (1-2ml) into a glass vial which evaporated till dryness (dry film).

Analytical Methods:

Determination of aflatoxins by HPLC-FLD:

By using High performance liquid chromatography (HPLC) Agilent model 1200 Infinity, the measurements were done in Chromatographic Lab, National Research Center, Giza, Egypt. The derivatives of positive samples and standards were done according to AOAC (2007).

Results and Discussion

Effect of different technological treatments on the aflatoxin levels in soybean seeds:

The results presented in Table (1) showed the effect of different technological treatments on the aflatoxin levels in soybean seeds.

Effect of fermentation treatment on the aflatoxin levels in soybean seeds:

Fermentation of raw soybean seeds that contain 50 µg/kg of AFB_1, AFB_2, AFG_1 or AFG_2 was accomplished by using pure strain of lactic acid bacteria (L.*casei* strain Lc12). The effect of fermentation treatment on the aflatoxin levels in tested soybean seeds is shown in Table (1).

From the Table, It could be observed that the fermentation treatment have an effect on lower of AFB_1 from 50.17 µg/kg for control sample (contaminated soybean seeds) to 34.6 µg/kg for fermented seeds with reduction percent about 31%, but it still be dangerous and unsafe for consumption because the AFB_1 residual in fermented soybean seeds is found at level largely above than 10 µg/kg which is the limiting value for human intake as reported by WHO (2006).

Based on that, the fermentation treatment is not effective as a method to prepare a safe raw soybean seeds for human intake. The partial removal of AFB_1 by fermentation was also observed by Haskard *et al.* (2001) who reported that viable cells of L. *lactis subsp lactis* and L. *casei Shirota* (YIT 901) remove 59 and 21.8% AFB_1, respectively. In a previous study by Megalla and Hafez (1982) they showed that the fermentation of yogurt and acidified milk contaminated with AFB_1 reduced the amount of the toxin but not capability remove all AFB_1 present.

The same Table shows that the control sample (contaminated soybean seeds) was free of AFB_2, while it containing AFG_1 and AFG_2 at levels 0.735 and 0.729 µg/kg, respectively. It is worth mentioning, the fermentation treatment completely eliminated AFG_1 and AFG_2 from tested soybean seeds (100% reduction). Abdellah *et al.* (2005) investigated the effect some strain of lactic acid bacteria on AFs degradation. They found that *Lactobacillus* strains could remove AFB_1 more than *Pediococcus* and *Leuconostoc* strains. The reduction of the initial amount of AFB_1 ranged from 1.80 to 44.89% for all strains studied. Five strains of *Lactobacillus rhamnosus*, and L. *casei* reduced AFB_1 by more than 20%. L. *rhamnosus* strain Lb50 reduced AFB_1 by 45% according to a study of Salem (2016).

Effect of germination treatment on the aflatoxin levels in soybean seeds:

The germination treatment is depended on the release of original enzymes in moistened seeds to digest their components (starch, protein…) to be more absorbed by intestine. The effect of germination on aflatoxins degradation for contaminated soybean seeds is shown in Table (1).

The results showed a weak effect for reduction of AFB_1 by germination treatment. In started soybean seeds that have 50.17 µg/kg of AFB_1 and exhibited level 44.9 µg/kg after germination with 10.5% reduction. Generally, AFB_1 residual after germination is greater than 10 µg/kg the limitary level by WHO (2006) to be accepted.

On the other hand, AFB_2, AFG_1 and AFG_2 were not detected in the tested seeds after germination treatment.

Effect of blanching treatment on the aflatoxin levels in soybean seeds:

The heating of soybean seeds by boiled water (cooking) is studied to know their effect on the removal of AFs in raw soybean seeds that artificially contaminated by AFB_1, AFB_2, AFG_1 and AFG_2. The remaining levels of AFs in soybean seeds after balancing are shown in Table (1).

The results showed that AFB_1 was averagely decreased from 50.17µg/kg to 38.2 µg/kg which affected by blanching in boiled water, with reduction % of 23.86%. The reduction of AFB_1 by blanching was also pointed out by Andrew *et al.* (2011), they reported that about 27% reduction of AFs when peanut seeds were blanched in boiling water. This means that such treatment was not significantly able to reduce aflatoxins to acceptable limit (10µg/kg).

It is worth mentioning, AFB_2 was not detected in the tested seeds after blanching treatment. Also, the same treatment completely eliminated AFG_1 and AFG_2 from these seeds (100% reduction).

Table 1: Effect of different technological treatments on aflatoxins reduction in soybean seeds

Afaltoxin	Control (µg/kg)	Fermented seeds		Germinated seeds		Blanched seeds		Roasted seeds	
		µg/kg	Reduction (%)	µg/kg	Reduction (%)	µg/kg	Reduction (%)	µg/kg	Reduction (%)
AFB_1	50.17	34.60	31.0	44.90	10.5	38.20	23.9	28.50	43.2
AFB_2	ND	ND	ND	ND	ND	ND	ND	ND	ND
AFG_1	0.735	ND	100	ND	100	ND	100	ND	100
AFG_2	0.729	ND	100	ND	100	ND	100	ND	100
Total	51.63	34.60	32.9	44.90	13.0	38.20	26.0	28.50	44.8

ND: Not detected

Effect of roasting treatment on the aflatoxin levels in soybean seeds:

Roasting treatment is normally diffused with soybean seeds and thus it is a necessary stage if oil has to be extracted from it. The effect of roasting treatment on AFs removal from contaminated soybean seeds was studied and the results are tabulated in Table (1).

The results showed that the roasting treatment reduced AFB_1 from 50.17 µg/kg to 28.5 µg/kg with reduction % of 43.2%. However, AFB_1 is still more than 10 µg/kg. Generally, these results are consistent with findings of Andrew *et al.* (2011).

On the other hand, AFB_2 was not detected in the tested seeds after roasting treatment. Also, the same treatment completely eliminated AFG_1 and AFG_2 from these seeds (100% reduction).

It can be concluded that the fermentation, germination, blanching and roasting treatments are not efficient to remove or degrade AFs from the tested seeds to the acceptable and recognized level (10 µg/kg) as reported by WHO (2014), where total reduction rates ranged from 13.0 to 44.8%. The germination treatment was the lowest effective among the tested treatments to remove aflatoxins from the soybean seeds.

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