Evaluation and detoxification of aflatoxins in ground and tree nuts using food grade organic acids

Farhat Jubeen[1], Farooq Sher[2*], Abu Hazafa[3], Fatima Zafar[4], Mariam Ameen[5], Tahir Rasheed[6]

[1]*Department of Chemistry, Government College Women University, Faisalabad 38000, Pakistan*

[2]*School of Mechanical, Aerospace and Automotive Engineering, Faculty of Engineering, Environment and Computing, Coventry University, Coventry CV1 5FB, UK*

[3]*Department of Biochemistry, University of Agriculture, Faisalabad, 38000, Pakistan*

[4] *Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore 54590, Pakistan*

[5]*Department of Chemical Engineering, Universiti Teknologi PETRONAS, Bandar Seri Iskandar, 31750, Tronoh, Perak, Malaysia.*

[6]*School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*

*Corresponding author: Email: Farooq.Sher@coventry.ac.uk (F.Sher)

Tel: +44 (0) 24 7765 7688

Abstract

The contamination of foodstuffs especially nuts with aflatoxins (AFs) affected by some of the fungal genera species that are a major threat to the economy, safe food supply, and serious health concerns to any country in recent days. Recently different techniques including heat, ozone, and microbes are used for the decontamination of aflatoxin but these all are limited to achieve the desirable results. The present study objectives to decontaminate the AFs in nuts by using three food-grade organic acids. In the present study, aqueous solutions of three food-grade organic acids namely citric, lactic and propionic acid are used at five different concentrations (1, 3, 5, 7 and 9%) to detoxify aflatoxin B_1 (AFB_1) and total aflatoxins (B_1, B_2, G_1, and G_2; TAFs) in selected nuts including almond, peanut, pistachio, and walnut at two different moisture levels (10±3 and 16±3%). The high-performance liquid chromatography (HPLC) coupled with fluorescence

detection method was applied for the qualitative and quantitative determination of AFs. The results showed that the decontamination of AFB_1 and total AFs significantly increased in infected nuts by increasing the concentration of acids. The experimental results of a 15 min treatment of walnut (10±3 and 16±3% moisture level), pistachio (10±3% moisture content) and peanuts (10±3% moisture content) with citric, lactic and propionic acids at 9% concentration significantly reduced of about 99.00, 99.90 and 96.07% of AFs respectively. Furthermore, treatment with citric and lactic acids resulted in the conversion of AFB_1 into less toxic products identified as AFD_1 via hydrolysis of the lactone ring. Citric acid was found as the most efficient acid in degrading the total AFs among all the three organic acids. The present study showed better AFs detoxification results than conventional methods. Hence, it is concluded that citric, lactic, and propionic acids can be applied as a useful and safe decontamination method of AFB_1 and total AFs in aflatoxin-affected nuts.

Keywords: Detoxification; Decontamination; Total aflatoxins; Aflatoxin B_1; Nuts; Citric acid.

1 Introduction

Mycotoxins are known as organic and low molecular weight secondary metabolites produced by several filamentous fungal genera, including *Fusarium*, *Aspergillus*, *Penicillium,* and *Alternaria.* These are toxic, which can cause diseases and deaths, both in humans and animals. According to a study of the Food and Agriculture Organization of the United Nations (FAO), mycotoxins contaminate approximately 25% of the world's crops each year (Akoto, Klu et al. 2017). To date, more than three hundred mycotoxins have been reported. However, just few mycotoxin associated compounds, including aflatoxin, zearalenone, deoxynivalenol, ochratoxin, and fumonisins are proved to be genotoxic, mutagenic and carcinogenic when they are present in food beyond the

limits set by FDA and IAEA (Yang 2019). Among these, aflatoxins (AFs) have received considerable attention during the past few decades because of their health impacts, including carcinogenic, teratogenic, and mutagenic potentials. Typically >20 different aflatoxin compounds have been investigated, but the aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) are the most prominent AFs that are ubiquitously reported in the dry food merchandise such as groundnuts, cereals, and spices (Ismail, Gonçalves et al. 2018).

Globally, nuts including walnut, almond, pistachio, and peanut are the most extensively cultivated crops that produce oil and essential components of other edible products. Because of their many outstanding beneficial health effects, the cultivation of nuts has been increased over the past few decades. The main challenge with the nuts production is contamination by AFs (Sukhotu, Guo et al. 2016, Abuagela, Iqdiam et al. 2019). Prevailing climatic conditions with increased moisture level and AFB_1 is recognized as the most potent carcinogenic compound in nuts and classified as Group 1 human carcinogen by the International Agency for Research on Cancer (IARC) that is associated with hepatocellular carcinoma (HCC), worldwide one of the common leading causes of deaths (Aiko, Edamana et al. 2016). The enzyme (cytochrome P-450) is responsible for the metabolism of AFB_1 to its reactive and carcinogenic metabolite, namely AFB_1-8,9-epoxide (AFBE) or its less responsive form like AFM_1, AFQ_1, or AFP_1 (Abrar, Anjum et al. 2013). Furthermore, with the increasing fact of diseases, the detoxification or degradation of AFs from food commodities has been necessary. Thermal, physical, and biological strategies have been investigated in connection with their effectiveness to prevent the foods from AFs contamination (Abuagela, Iqdiam et al. 2019).

Nevertheless, these processes showed the removal or degradation of AFs but due to their undesirable adverse effects and cost, these techniques have received less attention. Therefore, there is a need to develop a less expensive and more effective post-harvesting method to eliminate the fungus from the nuts. Thus, many food industries acknowledged the chemicals to degrade the AFB_1 into less toxic compounds (Wang, Mahoney et al. 2016). Ammonia is one of the commonly used chemicals for AFB_1 degradation by the corn industry that leads to the formation of less toxic and less mutagenic products as AFD_1. Similarly, chlorine gas has been accepted against AFB_1 by groundnut, copra, and cornmeal industries that successfully reduced 75% of AFB_1 without forming a hazardous compound (Aiko, Edamana et al. 2016). The chemicals such as acids, bases, bisulphites, oxidizing agents, and gases have been investigated against AFs contamination in peanut, cottonseed, and maize under suitable conditions (Pankaj, Shi et al. 2018). Citric acid is considered as a safe and edible food that has been successfully reported in the degradation of AFB_1 and showed of about 86 and 96.7% AFs reduction in the case of feeds and maize respectively (Méndez-Albores, Arambula-Villa et al. 2005, Méndez-Albores, Del Río-García et al. 2007). Chen and coworkers (2015) testified 100% inactivation of AFB_1 by using the lactic acid bacteria treatment. Moreover, Vandegraft et al. (1975) reported that 1% of propionic acid effectively inactive the toxic effect of AFB_1 in an artificially incubated corn up to 29 weeks of storage. According to another study, there was complete inhibition of aflatoxin biosynthesis in groundnut cake by using 0.5% concentration of propionic acid at room temperature (Ghosh, Chhabra et al. 1996).

Several types of researches are available in the literature on conventional techniques used in aflatoxin detoxification. But, the final measured endpoint in previous studies was the only

degradation of AFB_1; thus, the detoxified product was not explained. Moreover, the conversion of AFB_1 to AFB_{2a} as a detoxification step remained unknown. Therefore, an accurate and authentic study is required for the transformation of AFB_1 in a less toxic product that can efficiently weight to effectiveness of organic acids to degrade AFs. The present study aims to assess the potential benefits of three different types of organic acids, namely citric acid (CA), lactic acid (LA) and propionic acids (PA) for the degradation and detoxification of AFB_1 and total AFs in the selected nuts such as almond, peanuts, pistachio, and walnut. Moreover, the current investigations provide innovative facts on these types of food-grade organic acids to maximize the reduction of AFs that consequently could support the nuts industry without disturbing nuts quality.

2 Materials and methods

2.1 Chemicals

Aflatoxins (B_1, B_2, G_1, and G_2) and organic acids including citric acid (≥ 99.5%), lactic acid (≥ 98%) and propionic acid (≥ 95.5%) were obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA). Almonds, walnuts, pistachio, and peanuts were purchased from a local company (Faisalabad, Pakistan). Ethylene oxide (PubChem CID:6354; ≥ 99.7%), Acetonitrile (Pubchem CID:6342; ≥ 99%), Methanol (Pubchem CID:887; ≥ 99.85%), n-hexane (PubChem CID:8058; ≥ 99.9%), 2-propanol (Pubchem CID:3776; ≥ 99.5%) and all other chemicals used were reagent grade quality in the present study.

2.2 Fungal growth

Samples of tree nuts including almond (*Prunus duclus*), groundnut (*Arachis hyogea*), pistachio (*Pistachio vera*), and walnut (*Juglans regia*) were stored in glass containers. The samples were adjusted in two different moisture content levels (10±3% and 10±6%) with tap water. The samples

were kept under proper ventilation for a period of 2–3 hours daily up to 12 weeks. The levels of inherently contaminating mycoflora and aflatoxins were tested in 1st, 2nd, 4th and 12th week of storage. The samples were stored at 4±1 °C and randomly selected for the treatments from the stored lot. The initial moisture contents (MC) of walnut, almond, pistachio, and peanuts were found to be 0.38, 0.68, 0.54, and 0.71% respectively. Moisture contents were determined by drying replicate portions of groundnuts (5–10 g) at 106 °C for 24 h and subsequently up to constant weight. The loss in weight was expressed as the percentage and calculated on a wet weight by using Eq. (1) (USDA 1998).

$$\text{Moisture } \% = \frac{\text{Loss in weight of sample}}{\text{Weight of the sample}} \times 100 \quad (1)$$

The conditions for storage of nuts were adjusted according to Méndez-Albores et al. (2005) with little modifications. The moisture contents of the samples were modified to 10±3% and 16±3% with tap water and stored in wooden containers. To avoid any loss of moisture from nuts, the containers were roofed with polythene films. The accumulation of CO_2 generated by the respiration of nuts and expected fungal flora was prevented by making perforations approximately 10–20 times in the films. The containers were placed in a storeroom with proper aeration at 25–30 °C for 12 weeks. After the 12th week of storage, the nuts were placed under a 1000 mg ethylene oxide gas environment for 3 h to the hinder further multiplication of microorganisms. During 1st, 2nd, 4th and 12th weeks of storage, fungal growth and aflatoxin levels of ground and tree nuts for 10±3% and 16±3% moisture levels were regularly investigated. After a storage period of 12 weeks, the nuts were undertaken physical and chemical treatments for aflatoxin decontamination.

2.3 Chemical treatment of fungal nut samples

Chemical treatment of ground and tree nuts involved the use of organic acids. Three organic acids, namely citric acid (CA), lactic acid (LA), and propionic acid (PA) were employed at five different concentration levels including 1, 3, 5, 7, and 9% to evaluate the fungal decontamination and aflatoxin detoxification effects. Approximately 200–250 g samples of ground and tree nuts (stored for 12 weeks) were taken at two different moisture levels as 10±3% and 16±3% for chemical treatment. The acidification procedure of Méndez-Albores et al. (2007) was adopted with little modifications. Infected samples were placed in the form of a single film in wooden containers. Different concentrations of organic acids were exposed to samples at 1 mL/gm for a contact period of 15 min at room temperature (27±10 ^{o}C). The acid-treated samples of ground and tree nuts were filtered using a micro-fiber to take away surplus water and afterward dried in an oven at 30 ^{o}C for 4–5 h. The final moisture content was determined as reported previously. The contaminated and acid-treated samples were stored at 2±2 ^{o}C until further analysis.

2.4 Aflatoxins assay

2.4.1 AFs extraction and purification

Various extraction solvents can be used to study aflatoxins extraction and purification in the agriculture and food depending upon the requirements of the analyst. Chloroform extraction of aflatoxins presents excellent recoveries for composite commodities such as coffee and animal feed, but this method is very time-consuming. Methanol extraction is also used for aflatoxin analysis in nuts and cereals. Whereas acetonitrile extraction is particularly used for dried fruits and spices. The presence of a small amount of water in combination with an organic solvent humidifies the

substrate that increased the diffusion of organic solvent in the samples and resultingly increased the aflatoxin extraction.

The method for aflatoxins extraction in nut samples was according to the procedure reported by Liao et al. (2015) with little modifications. Samples of ground and tree nuts were randomly selected from the lot during the 1^{st}, 2^{nd}, 4^{th} and 12^{th} weeks of storage. Samples were grounded in a laboratory mill (Culatti, JANKE & KUNKEL, GmbH) and weighed 25 gm in Erlenmeyer flasks. Aflatoxins were extracted using 80 mL of a mixture of acetonitrile: water (84:16) by shaking for 30 min. The extract was filtered through Whatman (Maidatone, UK) filter paper (No. 3). From the filtrate, 9 mL was taken in a glass vial, acidified with 70 µL acetic acid and vortex. The acidified mixture was then passed through a mycosep # 226 Aflazon^{+} column (Romerlabs) with a flow rate of 2 mL/min. A pure aflatoxin solution (2 mL) was then dried through the stream of $N_{2,}$ and the residue was dissolved in a 2 mL of the mobile phase.

2.4.2 Derivatization and detection of AFs

The sensitivity of UV-vis detectors for AFs was up to ppm levels, whereas the fluorescent detector was up to ppb level. As AFB_1 and AFG_1 are less fluorescent, so post-column derivatization was carried out to convert into AFB_{2a} and $AFG_{2a,}$ respectively that are comparatively more fluorescent. Derivatization of AFG_1 and AFB_1 to AFG_{2a} and AFB_{2a} is a multistep process that was carried out using AOAC Method 990 which involves following steps: (1) First, the purified mixture (2 mL) of aflatoxins were taken in a glass vial to re-dissolve this purified mixture of aflatoxins, 200 µL hexane was added. (2) In the second step, 50 µL trifluoroacetic acid was added, then capped and vortex for 30 s, and allows for standing up to 5–6 min. (3) In the third step, 1.95 mL deionized

water was added into the water: acetonitrile (9:1) solution and vortex for 30 s, and it was allowed to stand for a while for the separation of two layers. (4) In the next step, the lower aqueous layer containing aflatoxins was removed and filtered through a 0.54 μm syringe filter tip. Finally, (5) the derivatized sample is ready for injection to HPLC.

2.4.3 Quantitative estimation of AFs

For qualitative and mainly quantitative evaluation of AFs, all analyses were performed on LC-system with following specifications: HPLC apparatus (Prominance™ , Shimadzu®, japan) containing Shimadzu LC software package designed for HPLC real-time and postoperative analysis operated through a computer equipped with Mediterranae Sea 18® 5 μm 25 cm x 0.46 Serial No. N45074 (Teknokroma, Spain) fitted with CTO-20A® (Shimadzu, Japan) column oven and LC-20AT® (Shimadzu, Japan) pump. The isocratic mobile phase consisting of methanol: acetonitrile: water (22.5: 22.5: 55) was used. The flow rate was maintained at 1 mL/min. Injection volume was 20 μL, Rheodyne® sample was injected with a 20 μL sample loop. The elute was detected by using spectrofluorometer detector RF-10A_{XL}® (Shimadzu, Japan) set at emission 440 nm and excitation at 360 nm.

2.4.4 Method validation parameters

Linearity was estimated by injecting AFB_1 with a triplicate standards concentration of 0.05, 0.1, 1, 5, 10, 20, 50, 100 and 150 ng/mL and 0.05, 0.1, 5, 10 and 20 ng/mL for AFG_1 triplicate standards. Similarly, the triplicate standard solutions of aflatoxin B_2 and G_2 at different concentrations as 0.02, 0.1, 1.5, 3 and 6 ng/mL for AFG_2 and 0.02, 0.03, 0.3, 1.5, 3, 6, 10 and 20 ng/mL for AFB_2 were injected. The recoveries were determined by spiking aflatoxins to control samples of nuts at concentration levels of 125.5 μg/kg for AFB_1, 15.3 μg/kg for AFG_1, and AFB_2, and 6.3 μg/kg for

206 AFG_2, which were calculated as 97.6, 91.2, 97.6, and 91.2% for AFB_1, AFB_2, AFG_1, and AFG_2
207 respectively. Triplicate samples were determined for each toxin level. The limit of detection and
208 limit of quantification was estimated based on signal to noise ratio as 3:1 for the limit of detection
209 (LOD) and 10:1 for the limit of quantification (LOQ), the values of LOD and LOQ for AFs are
210 presented in **Table 1**.

211 ## 2.5 Statistical Analysis

212 Three replicates of the fungal count, AFB_1, and total AFs were used, and all the analyses were
213 carried out in triplicates. Experimental data were subjected to analysis of variance (ANOVA:
214 α=0.05). Means (untreated vs treated) of each nut type were compared using t-test, statistical
215 package for the social sciences (SPSS) version IBM was used for this purpose.

216 # 3 Results and discussion

217 ## 3.1 Reduction of AFB_1 and total AFs in nuts by citric acid

218 The effect of different concentrations of aqueous citric acid such as 1, 3, 5, 7, and 9% on AFB_1
219 and total AFs (AFB_1, AFG_1, AFB_2, and AFG_2) in 12 weeks stored ground and tree nuts at two
220 moisture levels (10±3 and 16±3%) for 15 min treatment was studied. Citric acid significantly (P
221 <0.05) reduced the AFs levels in the selected nuts. The maximum reduction of 99 and 97% for
222 AFB_1 and total AFs were found in walnuts treated with 9% aqueous citric acid for 15 min treatment
223 both at high and low moisture contents (10±3 and 16±3%). In these samples, the levels of AFB_1
224 and total AFs were reduced from 0.08 ± 0.02 and 0.14 ± 1.80 to 0.03 ± 0.01 and 0.05 ± 1.50 μg/kg
225 at low and high moisture contents, respectively. The AFB_1 reduction at both moisture levels is

represented in **Fig. 1.** In the presence of citric acid after 20 min treatment, 98% reduction of AFB_1 in contaminated feed was estimated by Rushing and Selim (2016).

Similarly, >95% reduction in total AFs was expected by Jubeen et al. (2012) in peanuts when peanuts were treated with UV radiation for 45 min. In peanuts, the final levels of AFB_1 and total AFs by using 9% citric acid concentration were 2.29 ± 0.10 and 2.42 ± 0.60 μg/kg at low moisture level, and 7.29 ± 1.05 and 7.56 ± 1.30 μg/kg at high moisture content respectively and 2.28±0.3 and 2.29±0.4 μg/kg in pistachio adjusted at high moisture level. In these samples, the final levels of AFB_1 and total AFs at the highest citric acid concentration (9%) were beyond the regulatory limit of 2 μg/kg set by IAEA, WHO, and FDA. While in the rest of the samples both at low and high moisture contents, the final levels of AFB_1 were found below 2 μg/kg at the highest citric acid concentration, but in total AFs the levels were found above the 2 μg/kg at the highest citric acid concentration except for walnut. This was observed that the food matrix also affects the detoxification efficiency of the chemical reagent which is also consistent with previous studies (Méndez-Albores, Arambula-Villa et al. 2005, Mendez-Albores, Veles-Medina et al. 2009, Rastegar, Shoeibi et al. 2017).

The results of previously published literature are in agreement with our findings, the experiment of Rastegar et al. (2017) reported a 93.1% reduction of AFB_1 in pistachio nuts by citric acid treatment (6 g) at 120 ℃/1 h. Safara et al. (2010) recorded a 97.22% reduction of AFB_1 in rice by (I N) citric acid treatment for 15 min. Similarly, applying the same amount of citric acid (1 N) and time (15 min), 96.70% degradation of B-aflatoxins (AFB_1+AFB_2) in maize (Méndez-Albores, Arambula-Villa et al. 2005), 92% in sorghum (Méndez-Albores, Martínez-Bustos et al. 2008), and

86% of duckling feed (Méndez-Albores, Del Río-García et al. 2007) was estimated. The results are also similar to those obtained by Savi et al. (2015), 81–95% reduction in AFs in wheat was observed when wheat was treated with ozone for 30–180 min at 40–60 mg/L concentrations. The estimated coefficients (β_0 and β_1) for exponential decay functions of AFB_1 are presented in **Table 2**. The estimated coefficients (β_0 and β_1) for nuts at different moisture levels were determined by using the following Eq. (2) (Mendez-Albores, Veles-Medina et al. 2009):

$$y = \beta_0 \exp(\beta_1 x) + \varepsilon \quad (2)$$

Where y represents the concentration of aflatoxin (%), β_0 and β_1 are the estimated coefficients, x represents the amount of acids in the samples (μg/kg), and ε is the experimental error. The effect of adding different concentrations of citric acid on total AFs degradation fits with an exponential decay function is represented in **Fig. 2**. The effect of different citric acid concentrations on total AFs content in nuts revealed that the total AFs were below than 4 μg/kg except in peanut at both moisture levels. There was 7.56 μg/kg AFs content in peanut at 16±3% moisture level.

3.1.1 Degradation mechanism by citric acid

It is proposed that detoxification of AFB_1 and total AFs initially involves the acid-catalyzed hydrolysis of the lactone ring resulting in the formation of β-keto acid structure, which upon decarboxylation, formed a new molecule named as AFD_1 (**Fig. 3**) (Méndez-Albores, Arambula-Villa et al. 2005). The mutagenic character of AFD_1 is 450 times less than AFB_1 which presents 18-fold less toxicity (Nicolás-Vázquez, Méndez-Albores et al. 2010). It is reported that charge transference in the lactone ring and on some carbon atom of benzene indicates the existence of some conjugation among them. The charge transfer observed between the ground and the excited

singlet state showed fluorescence and a reduction in the electronic charge of the atoms involved in the lactone ring. Therefore, the fluorescence phenomenon diminishes when the AFs structure is hydrolyzed. The proposed reaction mechanism of AFB_1 acidification has also been confirmed by both MS/MS and computational studies (Méndez-Albores, Martínez-Bustos et al. 2008, Jardon-Xicotencatl, Díaz-Torres et al. 2015).

Furthermore, the Ames test suggests that the difuran structure undergo some alterations after treatment. Aqueous citric acid caused hydration of 8, 9-double bond of terminal furan ring in AFB_1 to form hydroxydihydro-aflatoxin B_1 (AFB_2a), it has about 200 times less toxicity than AFB_1 (Rushing and Selim 2017). AFB_1 differs from AFB_2 in the existence of 8,9-double bond at the terminal furan ring, and this small difference in structure is responsible for a momentous change in the activity. However, AFB_1 is carcinogenic and noticeably more toxic than AFB_2. So, an obvious moiety in the detoxification of AFB_1 is the vinyl ether double bond. Catalytic hydration of the bond using mineral acid and trifluoroacetic acid has been shown to produce hemiacetal aflatoxins (Yazdanpanah and Eslamizad 2015, Rushing and Selim 2016).

3.2 Inactivation of AFB1 and total AFs by lactic acid

Lactic acid selectively affected AFB_1 and total AFs in 12-weeks stored nuts. The level of AFB_1 reduced significantly with a concordant increase in AFB_2 in almost all nuts (walnut, peanut, pistachio, and almond) treated by lactic acid at different concentrations as 1, 3, 5, 7, and 9% with 15 min treatment. Lactic acid significantly ($P < 0.05$) reduced the AFs levels in selected nuts including walnut, peanut, pistachio, and almond. The percent decrease of AFB_1 in 12-weeks stored walnuts (10±3% moisture content) treated with different concentrations of aqueous lactic acid are

54.30, 77.40, 84.80, 90.80, and 95.50 μg/kg after 15 min treatment as shown in **Fig. 4**. Interestingly, in the same samples of walnut, an increase in AFB_2 at similar concentrations of lactic acid is redetermined as 48.40, 65.20, 73.50, 86.60, and 91.30 μg/kg reduction. Although improvement of AFB_2 is not in the same proportion as a decrease in AFB_1 but no irregularity was seen in this pattern of increase and decrease. The observations recorded are consistent with (Méndez-Albores, Martínez-Bustos et al. 2008), who stated up to a 67% reduction of B-aflatoxins (AFB_1 and AFB_2) with aqueous lactic acid at sorghum flour (30% MC and 8 mol/L lactic acid). The results are also similar to those obtained by Aiko et al. (2016) who reported different molar concentrations of lactic acid (0.1, 0.5, and 1 mol/L) and heating temperature of 37, 50, and 80 ℃ showed an increasing concentration of acid result in increasing the efficiency of lactic acid to degrade the AFB_1 into AFB_2.

Levels of AFB_1 and total AFs in nuts both at low and high moisture contents reduced significantly. The maximum reduction (99.9 and 94.5%) of AFB_1 and total AFs was observed in pistachio and walnut (10±3% moisture content) respectively, by using 9% lactic acid treatment for 15 min. The results are similar to those obtained by Lee et al. (2015), who reported that the lactic acid treatment with the concentration of 1 N for 18 h significantly reduces the 93% of AFs in soybeans. These findings are in agreement with (Méndez-Albores, Martínez-Bustos et al. 2008). These results are also supported by the studies of Mousavi-Khaneghah et al. (2018), who reported that the binding of aflatoxins with lactic acid bacteria is extracellular and to improve intracellular binding bacteria should be acid-treated, and his study suggested that the use of lactic acid bacteria such as *L. casei* and *L. plantarum* could significantly reduce AFB_1 in maize. The investigation fluctuates from Mousavi-Khaneghah et al. (2018) in the use of lactic acid directly instead of lactic acid bacteria

for detoxification of aflatoxins. But the present study justified in the choice of using lactic acid rather than lactic acid bacteria as they are notoriously known to produce their toxins, by the decarboxylation of the amino acids present in the substrate, known as biogenic amines (Zuljan, Mortera et al. 2016, Gloria and Engeseth 2019).

Ahlberg et al. (2015) investigated a model system to estimate the AFB_1 binding capacity of lactic acid bacteria, where no substrate was available for toxin formation. Many studies (Ahlberg, Joutsjoki et al. 2015, Bovo, Franco et al. 2015, Chen, Kong et al. 2015) are reported on using various strains of lactic acid bacteria in model systems to bind aflatoxins with fungi that are responsible for aflatoxin formation. In the present work, although lactic acid significantly reduced AFB_1 and total AFs that are potentially more toxic than AFB_2 and AFG_2, but with a due increase in the levels of AFB_2 and AFG_2. As a result, the total AFs content did not fall up to maximum tolerable limits. In walnuts, at both moisture levels (10±3% and 16±3%) treated with 9% lactic acid showed total AFs content of 2.26±0.3 and 4.06±0.1 μg/kg. In the rest of the samples, total AFs content was quite high even at 9% lactic acid treatment as shown in **Fig. 5.**

Lactic acid treatment for aflatoxin detoxification is preferable than using lactic acid bacteria because no pretreatment of the samples or adding reagent is required. Only aqueous solutions at different concentrations are used. It is a time-saving method for aflatoxin detoxification. The maximum reduction of AFB_1 and total AFs in our analyzed samples were approximately 99 and 94.5% in a treatment time of only 15 min at a concentration of 9%, and no shaking was carried out in this process. However, Chen et al. (2015) reported a maximum of 100% decontamination of AFB_1 by using the mixed treatment of *Streptococcus thermophilus* and *L. delbrueckii* subsp.

339 *Bulgaricus* on pistachio nuts. The test culture of bacterial strain used in this study was also subbed
340 cultured three times on aflatoxin containing medium for possible induction of enzyme responsible
341 for aflatoxin degradation.
342
343 Similarly, Silva et al. (2015) reported a 96% reduction in total AFs in peanut with *A. parasiticus*,
344 when it was combined with *Lactobacillus delbrueckii*. However, Farzaneh et al. (2012) reported
345 that 95% removal of AFB_1 in nuts by a selected strain of lactic acid bacteria (*Bacillus subtilis*;
346 UTBSP1) by a rapid process. The percentage of AFB_1 residue at 0 h was not different from that at
347 72 h, suggesting that the elimination of aflatoxin is a rapid process. The use of lactic acid instead
348 of bacterial strains is free from all these constraints, time effective, applicable without the chance
349 of an increase in the microbial population of food or feed as well as economical. The risk of culture
350 contamination is always there, which may alter the desired results. The effect of adding different
351 concentrations of lactic acid on AFB_1 and total AFs degradation fit with an exponential decay
352 function, as shown in **Fig. 4** and **Fig. 5**, respectively, at both low and high moisture levels. The
353 estimated coefficients **(**β_0 and β_1) for exponential decay functions of AFB_1 are presented in **Table**
354 **3.** The values of estimated coefficients **(**β_0 and β_1) for aflatoxin degradation by lactic acids on
355 different nuts were determined by using Eq. (2).

356 ### 3.2.1 Detoxification mechanism of AFs by lactic acid

357 The increase in AFB_2 may be due to the structural changes in AFB_1, leading to their conversion
358 into AFB_2. It may be due to the fact that lactic acid does not affect AFB_2 residues already present
359 in 12-week stored ground and tree nuts. This observation is the following (Méndez-Albores,
360 Arambula-Villa et al. 2005, Aiko, Edamana et al. 2016**).** The proposed mechanisms for the
361 conversion of AFB_1 into AFB_2 is shown in **Fig. 6**. The enzymatic reaction involving biochemical

oxidation of lactic acid to pyruvic acid by lactate dehydrogenase is well known in metabolic pathways. In this process, NAD is reduced to $NADH_2$, suggesting an overall shift of two protons and two electrons from lactic acid. A structural analogue of lactate dehydrogenase and ascorbate dehydrogenase also seems to be efficient in reducing AFB_1 to corresponding fewer toxic products as AFB_2. The proposed mechanism for the reduction of AFB_1 to AFB_2 involves the initial formation of transient oxonium intermediate, which tends to polarize the olefinic (C=C) carbon. It causes hydride abstraction from the α-carbon of lactic acid. Here, the overall process involves the transfer of two protons from lactic acid to AFB_1. Pyruvic acid is the oxidation product of lactic acid (Shukla, Verma et al. 2002).

3.3 Reduction of AFB_1 and total AFs in nuts by Propionic acid

The result of different concentrations (1, 3, 5, 7, and 9%) of the propionic acid on AFB_1 and total AFs were studied in-ground and tree nuts, following a storage period of 12-weeks at two different moisture levels with 15 min treatment. Propionic acid significantly ($P < 0.05$) reduced the AFs levels in selected nuts. The results indicated that increasing concentrations of propionic showed a substantial decrease in individual as well as total AFs levels. The working mechanism behind aflatoxin reduction is still unrevealed. In nuts adjusted at low moisture content, the level of AFs was more moderate than those at higher moisture levels. As a result of propionic acid treatment, the nuts, which are presenting total AFs quantity lower than 4 ppb and AFB_1 smaller than 2 ppb are those that were adjusted at lower moisture levels.

Fig. 7 showed that walnut at both the moisture levels after 9% propionic acid treatment revealed aflatoxin contents below the maximum tolerable limits. Almond and pistachio at low moisture

level after treatment with 9% propionic acid showed total AFs content smaller than 4 μg/kg. In the case of peanut adjusted at lower moisture level, the level of AFB_1 and total AFs reduced from 46.78, and 51.80 μg/kg to 17.99, and 22.19 μg/kg after treatment with 1% propionic acid with percentage degradation of 61.55 and 67%, respectively. The level of AFB_1 and total AFs reduced proportionally by increasing the propionic acid concentration, and the maximum reduction ratio was determined as 96.07 and 91% in peanut at 9% propionic and residual AFB_1 and total AFs levels were 1.83 and 5.55 μg/kg, respectively. This observation is followed Molina and Giannuzz (2002).

Peanuts adjusted at a high moisture level showed a 95.78% AFB_1 reduction with the final aflatoxin level of 6.68 μg/kg. The highest reduction, approximately 99% of AFB_1, was observed in walnuts at both the moisture levels. But in the case of total AFs, the maximum reduction of about 96% was achieved in walnut with the concentration of 9% at low moisture level and the final sample level was 0.299 ng/g, while at high moisture level at same concentration the reduction ratio was 94% in walnut after 15 min treatment and the final aflatoxin level was 0.498 ng/g. The results of AFB_1 reduction by propionic acid are in agreement with Hasan (1996), who reported more than 90% reduction of AFB_1 in sorghum using propionic acid. It was found that AFG_2 was not detected in any nut sample treated with the lowest propionic acid concentration and afterward. After treatment with propionic acid, AFG_1 was detected only in walnut and peanut at high moisture levels and almond at both the moisture levels, while in the rest of the samples, AFG_1 was eliminated. However, it is reported that AFG_2 and AFB_2 has a little resistant to acid treatment (Abbas, Weaver et al. 2005). But the complete degradation of AFG_2 by propionic acid may be due to its low initial concentration.

Molina and Giannuzzi (1999) revealed that a linear relationship exists between the lag phase and the reciprocal growth rate at different propionic acid concentrations. The effect of adding different concentrations of propionic acid on aflatoxin degradation was found to fit with an exponential decay function (Méndez-Albores, Martínez-Bustos et al. 2008). The values of estimated coefficients (β_0 and β_1) calculated for AFB_1 in nuts for different propionic acid concentrations are presented in **Table 4**. These estimates provide the theoretical basis for aflatoxin degradation for propionic acid concentrations beyond the scale of our observed levels. But under different conditions such as pH, concentration, and type of commodity, these values will be different. As these values are estimated at 95% confidence interval, it means that we are 95% confident about the set of our employed experimental conditions, the estimated values of β_0 and β_1 fall in the reported range. The effect of adding different concentrations of propionic acid on total aflatoxins degradation fits with an exponential decay function is represented in **Fig. 8.**

The present study considered three different types of organic acids including citric, lactic, and propionic acids for the degradation and detoxification of AFB_1 and total AFs in selected nuts (almond, peanuts, pistachio, and walnut) due to their high degradation ability and cost-effectiveness. The results showed more than 99% decontamination of total AFs by citric acid treatment over 15 min exposure in walnut with 9% concentration. Furthermore, 99.90 and 96.07% detoxification of AFs accomplished by lactic acid and propionic acid under the same reaction conditions in pistachio and peanut respectively, as presented in **Table 5**. Our findings are in correlation with the results of Hojnik et al. (2019), they reported that 8 min treatment of cold atmospheric pressure plasma (CAP) significantly removes over 93% AFs in foods. Similarly, Savi

et al. (2015) revealed that 30–180 min exposure of ozone (40–60 mg/L) effectively reduces 81–95% of total aflatoxins (B_1, B_2, G_1, and G_2) in wheat. Moreover, Siciliano and his research group stated that 40 min exposure of infrared rays successfully removes more than 80% of total AFs in Turkish hazelnuts (Siciliano, Dal Bello et al. 2017).

The data revealed that the treatment of AF with organic acids showed better results than the conventionally used AF detoxification methods including roasting, microbes, ozone, and cold plasma treatments. Therefore, the organic acids could be a viable option for the treatment of AFs in the near future.

4 Conclusions

Among all mycotoxins, the AFs have received considerable attention because of their severe health impacts. Detoxification and decontamination of AFs remain a challenge for the food factories. Three acids (citric, lactic, and propionic acid) have been found useful for significant aflatoxins degradation in-ground and tree nuts adjusted at two different moisture levels. Data revealed that citric acid showed a considerable reduction in all the four aflatoxins including AFB_1, AFG_1, AFB_2, and AFG_2 without the formation of any hazardous residues. The maximum aflatoxin decontamination of about 99.00% (walnut), 99.90% (pistachio), and 96.07% (peanut) was found at 9% concentration with citric, lactic, and propionic acids respectively after 15 min treatment. Lactic acid significantly reduced AFB_1 and total AFs with a concordant increase in AFB_2 and AFG_2. It is justified that lactic acid brought about the reduction of AFB_1 by its conversion into less toxic AFB_2. Propionic acid was found more efficient in reducing AFB_1 but quite less reducing efficiency was found in AFB_2 and AFG_2. However, the use of organic acids including citric, lactic, and propionic acids could be a viable AFs decontamination option for the future. Moreover, further

452 study studies are required to understand the mechanism of action of food graded organic acids and
453 their effects on food merchandise.

454 Acknowledgement

455 The authors are thankful to the Higher Education Commission of Pakistan for the financial support
456 to carry on this research [project number 8966/Federal/NRPU/R&D/2017].
457

References

Abbas, H., M. Weaver, R. Zablotowicz, B. Horn and W. T. Shier (2005). "Relationships between aflatoxin production and sclerotia formation among isolates of Aspergillus section Flavi from the Mississippi Delta." European Journal of Plant Pathology 112(3): 283-287.

Abrar, M., F. M. Anjum, M. S. Butt, I. Pasha, M. A. Randhawa, F. Saeed and K. Waqas (2013). "Aflatoxins: biosynthesis, occurrence, toxicity, and remedies." Critical reviews in food science nutrition 53(8): 862-874.

Abuagela, M. O., B. M. Iqdiam, H. Mostafa, S. M. Marshall, Y. Yagiz, M. R. Marshall, L. Gu and P. Sarnoski (2019). "Combined effects of citric acid and pulsed light treatments to degrade B-aflatoxins in peanut." Food Bioproducts Processing 117: 396-403.

Ahlberg, S. H., V. Joutsjoki and H. J. Korhonen (2015). "Potential of lactic acid bacteria in aflatoxin risk mitigation." International journal of food microbiology 207: 87-102.

Aiko, V., P. Edamana and A. Mehta (2016). "Decomposition and detoxification of aflatoxin B1 by lactic acid." Journal of the Science of Food Agriculture 96(6): 1959-1966.

Akoto, E., Y. Klu, M. Lamptey, J. Asibuo, J. Davis, R. Phillips, D. Jordan, J. Rhoads, D. Hoistington and J. Chen (2017). "Use of peanut meal as a model matrix to study the effect of composting on aflatoxin decontamination." World Mycotoxin Journal 10(2): 131-141.

Bovo, F., L. T. Franco, R. E. Rosim, R. Barbalho and C. A. F. d. Oliveira (2015). "In vitro ability of beer fermentation residue and yeast-based products to bind aflatoxin B1." Brazilian Journal of Microbiology 46(2): 577-581.

Chen, Y., Q. Kong, C. Chi, S. Shan and B. Guan (2015). "Biotransformation of aflatoxin B1 and aflatoxin G1 in peanut meal by anaerobic solid fermentation of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus." International journal of food microbiology 211: 1-5.

Farzaneh, M., Z.-Q. Shi, A. Ghassempour, N. Sedaghat, M. Ahmadzadeh, M. Mirabolfathy and M. Javan-Nikkhah (2012). "Aflatoxin B1 degradation by Bacillus subtilis UTBSP1 isolated from pistachio nuts of Iran." Food control 23(1): 100-106.

Ghosh, M., A. Chhabra, P. Atreja and R. Chopra (1996). "Effect of treating with propionic acid, sodium bisulfite and sodium hydroxide on the biosynthesis of aflatoxin on groundnut cake." Animal feed science technology 60(1-2): 43-49.

Gloria, M. B. A. and N. J. Engeseth (2019). "Bioactive amines."

Hasan, H. (1996). "Destruction of aflatoxin B1 on sorghum grain with acids, salts and ammonia derivatives." Cryptogamie Mycologie.

Hojnik, N., M. Modic, G. Tavčar-Kalcher, J. Babič, J. L. Walsh and U. Cvelbar (2019). "Mycotoxin Decontamination Efficacy of Atmospheric Pressure Air Plasma." Toxins 11(4): 219.

Ismail, A., B. L. Gonçalves, D. V. de Neeff, B. Ponzilacqua, C. F. Coppa, H. Hintzsche, M. Sajid, A. G. Cruz, C. H. Corassin and C. A. Oliveira (2018). "Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination." Food Research International 113: 74-85.

Jardon-Xicotencatl, S., R. Díaz-Torres, A. Marroquín-Cardona, T. Villarreal-Barajas and A. Méndez-Albores (2015). "Detoxification of aflatoxin-contaminated maize by neutral electrolyzed oxidizing water." Toxins 7(10): 4294-4314.

Jubeen, F., I. A. Bhatti, M. Z. Khan, H. Zahoor-Ul and M. Shahid (2012). "Effect of UVC irradiation on aflatoxins in ground nut (Arachis hypogea) and tree nuts (Juglans regia, Prunus duclus and Pistachio vera)." Journal of the Chemical Society of Pakistan 34(6): 1366-1374.

Lee, D., J. Lyu and K.-G. Lee (2015). "Analysis of aflatoxins in herbal medicine and health functional foods." Food Control 48: 33-36.
Liao, C.-D., J. W. Wong, K. Zhang, P. Yang, J. B. Wittenberg, M. W. Trucksess, D. G. Hayward, N. S. Lee and J. S. Chang (2015). "Multi-mycotoxin analysis of finished grain and nut products using ultrahigh-performance liquid chromatography and positive electrospray ionization–quadrupole orbital ion trap high-resolution mass spectrometry." Journal of agricultural food chemistry 63(37): 8314-8332.
Méndez-Albores, A., G. Arambula-Villa, M. Loarca-Piña, E. Castano-Tostado and E. Moreno-Martínez (2005). "Safety and efficacy evaluation of aqueous citric acid to degrade B-aflatoxins in maize." Food chemical toxicology 43(2): 233-238.
Méndez-Albores, A., J. Del Río-García and E. Moreno-Martinez (2007). "Decontamination of aflatoxin duckling feed with aqueous citric acid treatment." Animal feed science technology 135(3-4): 249-262.
Mendez-Albores, A., J. Veles-Medina, E. Urbina-Alvarez, F. Martinez-Bustos and E. Moreno-Martínez (2009). "Effect of citric acid on aflatoxin degradation and on functional and textural properties of extruded sorghum." Animal feed science technology 150: 316-329.
Méndez-Albores, A., F. Martínez-Bustos, M. Gaytán-Martínez and E. Moreno-Martínez (2008). "Effect of lactic and citric acid on the stability of B-aflatoxins in extrusion-cooked sorghum." Letters in applied microbiology 47(1): 1-7.
Mohamed, N. F., R. S. S. El-Dine, M. A. M. Kotb and A. Saber (2015). "Assessing the possible effect of gamma irradiation on the reduction of aflatoxin B1, and on the moisture content in some cereal grains." American Journal of Biomedical Science 7(1): 33-39.
Molina, M. and L. Giannuzzi (1999). "Combined effect of temperature and propionic acid concentration on the growth of Aspergillus parasiticus." Food research international 32(10): 677-682.
Molina, M. and L. Giannuzzi (2002). "Modelling of aflatoxin production by Aspergillus parasiticus in a solid medium at different temperatures, pH and propionic acid concentrations." Food Research International 35(6): 585-594.
Mousavi Khaneghah, A., I. Eş, S. Raeisi and Y. Fakhri (2018). "Aflatoxins in cereals: State of the art." Journal of food safety 38(6): e12532.
Nicolás-Vázquez, I., A. Méndez-Albores, E. Moreno-Martínez, R. Miranda and M. Castro (2010). "Role of lactone ring in structural, electronic, and reactivity properties of aflatoxin B1: A theoretical study." Archives of environmental contamination toxicology 59(3): 393-406.
Pankaj, S., H. Shi and K. M. Keener (2018). "A review of novel physical and chemical decontamination technologies for aflatoxin in food." Trends in Food Science Technology 71: 73-83.
Porto, Y. D., F. M. Trombete, O. Freitas-Silva, I. M. De Castro, G. M. Direito and J. L. R. Ascheri (2019). "Gaseous Ozonation to Reduce Aflatoxins Levels and Microbial Contamination in Corn Grits." Microorganisms 7(8): 220.
Rastegar, H., S. Shoeibi, H. Yazdanpanah, M. Amirahmadi, A. M. Khaneghah, F. B. Campagnollo and A. S. Sant'Ana (2017). "Removal of aflatoxin B1 by roasting with lemon juice and/or citric acid in contaminated pistachio nuts." Food control 71: 279-284.
Rushing, B. R. and M. I. Selim (2016). "Effect of dietary acids on the formation of aflatoxin B2a as a means to detoxify aflatoxin B1." Food Additives Contaminants: Part A 33(9): 1456-1467.

Rushing, B. R. and M. I. Selim (2017). "Structure and oxidation of pyrrole adducts formed between aflatoxin B2a and biological amines." Chemical research in toxicology 30(6): 1275-1285.
Safara, M., F. Zaini, S. J. Hashemi, M. Mahmoudi, A. R. Khosravi and F. Shojai-Aliabadi (2010). "Aflatoxin detoxification in rice using citric acid." Iranian journal of public health 39(2): 24.
Savi, G. D., K. C. Piacentini and V. M. Scussel (2015). "Ozone treatment efficiency in Aspergillus and Penicillium growth inhibition and mycotoxin degradation of stored wheat grains (Triticum aestivum L.)." Journal of food processing preservation 39(6): 940-948.
Shukla, R. S., R. J. Verma and D. N. Mehta (2002). "Kinetic and mechanistic investigations on reductions of aflatoxins by lactic acid." Bioorganic medicinal chemistry letters 12(19): 2737-2741.
Siciliano, I., B. Dal Bello, G. Zeppa, D. Spadaro and M. L. Gullino (2017). "Static hot air and infrared rays roasting are efficient methods for aflatoxin decontamination on hazelnuts." Toxins 9(2): 72.
Silva, J. F. M. d., J. M. Peluzio, G. Prado, J. E. G. C. Madeira, M. O. Silva, P. B. de Morais, C. A. Rosa, R. S. Pimenta and J. R. Nicoli (2015). "Use of probiotics to control aflatoxin production in peanut grains." The Scientific World Journal 2015.
Sukhotu, R., S. Guo, J. Xing, Q. Hu, R. Wang, X. Shi, K. Nishinari, Y. Fang and S. Guo (2016). "Changes in physiochemical properties and stability of peanut oil body emulsions by applying gum arabic." LWT-Food Science Technology 68: 432-438.
USDA (1998). The Official United States Standards for Grain. Washington, DC.
Vandegraft, E., C. Hesseltine and O. Shotwell (1975). "Grain preservatives: Effect on aflatoxin and ochratoxin production." Cereal Chem 52(1): 79.
Wang, B., N. E. Mahoney, Z. Pan, R. Khir, B. Wu, H. Ma and L. Zhao (2016). "Effectiveness of pulsed light treatment for degradation and detoxification of aflatoxin B1 and B2 in rough rice and rice bran." Food Control 59: 461-467.
Yang, Q. (2019). Decontamination of Aflatoxin B1. Aflatoxin B1 Occurrence, Detection and Toxicological Effects, IntechOpen.
Yazdanpanah, H. and S. Eslamizad (2015). "Aflatoxins and their management." Biological Toxins Bioterrorism: 103-120.
Zhang, G., J. Li, J. Lv, L. Liu, C. Li and L. Liu (2019). "Decontamination of aflatoxin M1 in yogurt using Lactobacillus rhamnosus LC-4." Journal of Food Safety 39(5): e12673.
Zuljan, F. A., P. Mortera, S. H. Alarcón, V. S. Blancato, M. Espariz and C. Magni (2016). "Lactic acid bacteria decarboxylation reactions in cheese." International Dairy Journal 62: 53-62.

List of Tables

589 **Table 1**. Validation of aflatoxin determination by HPLC analysis.

Aflatoxin	LOD (ng/mL)	LOQ (ng/mL)	Calibration curve	R^2	Recovery (%)	Mean (µg/kg) ± RSD (%)
AFB_1	0.02	0.05	y = 68983x + 34942	0.9997	97.6	125.3 ± 9.12
AFB_2	0.01	0.02	y = 104767x - 6094	0.9995	91.2	15.3 ± 2.01
AFG_1	0.02	0.05	y = 32045x + 2780	0.9996	97.6	15.3 ± 1.44
AFG_2	0.01	0.02	y = 61801x - 85618	0.9991	91.2	6.3 ± 3.42

590

591

Table 2. The estimated coefficient for the exponential decay equation of AFB_1 by citric acid in tree and ground nuts at different moisture levels.

Moisture contents (%)	Types of nuts	95% confidence interval			
		β_0	β_1	β_0'	β_1'
16±3	Walnut*	(2.34, 30.10)	(-0.49, 0.03)	16.21	-0.23
	Almond*	(16.49, 40.12)	(-0.59, 0.14)	28.31	-0.36
	Pistachio*	(17.61, 39.09)	(-0.57, 0.16)	28.35	-0.37
	Peanut*	(1.92, 4.41)	(-0.06, -0.17)	3.17	-0.04
10±3	Walnut	(3.63, 71.55)	(-1.19, 0.10)	37.59	-0.54
	Almond	(17.61, 39.09)	(-0.79, -0.26)	43.33	-0.52
	Pistachio	(33.09, 80.67)	(-1.19, 0.28)	56.88	-0.73
	Peanut	(28.92, 174.57)	(-2.68, 0.09)	101.75	-0.29

595 **Table 3.** Estimated coefficients for the exponential decay equation of AFB_1 by lactic acid in tree
596 and ground nuts at different moisture levels.
597

Moisture contents (%)	Types of nuts	95% confidence interval			
		β_0	β_1	β_0'	β_1'
16±3%	Walnut*	(17.97, 68.97)	(-0.35, -0.62)	43.47	-5.49
	Almond*	(11.55, 58.85)	(-9.18, 0.16)	35.20	-4.67
	Pistachio*	(15.11, 83.36)	(-3.38, -0.37)	49.23	-6.88
	Peanut*	(34.39, 175.54)	(-27.06, 0.144)	104.96	3.60
10±3%	Walnut	(7.56, 29.12)	(-4.36, -0.25)	18.34	-2.31
	Almond	(7.70, 39.39)	(-6.14, -0.09)	23.55	-3.11
	Pistachio	(7.28, 39.02)	(-6.30, -0.24)	23.15	-3.27
	Peanut	(9.81, 51.74)	(-7.99, 0.01)	30.78	-3.99

598

Table 4. Estimated coefficients for the exponential decay equation of AFB_1 by propionic acid in tree and ground nuts at different moisture levels.

Moisture contents (%)	Types of nuts	95% confidence interval			
		β_0	β_1	β_0'	β_1'
16±3%	Walnut*	(3.05, 70.56)	(-0.16, 0.12)	36.81	-0.52
	Almond*	(14.42, 57.86)	(-0.88, 0.05)	36.15	-0.46
	Pistachio*	(7.59, 81.13)	(-0.30, 0.10)	44.36	-0.60
	Peanut*	(33.68, 174.30)	(-2.68, 0.00)	103.99	-0.34
10±3%	Walnut	(4.09, 29.68)	(-0.48, 0.01)	16.89	-0.23
	Almond	(7.19, 39.66)	(-0.62, -0.00)	23.42	-0.31
	Pistachio	(3.99, 37.77)	(-0.60, -0.04)	20.88	-0.28
	Peanut	(10.33, 51.33)	(-0.79, -0.01)	30.83	-0.40

Table 5. The aflatoxin detoxification comparison between conventional degradation methods and present study reported organic acids (citric, lactic, and propionic acids).

Aflatoxins	Food stuffs	Degradation (%)	Treatment time (min)	Analytical method	Degradation method	Reference
TAFs	Pistachio	99.90	15.00	HPLC	Lactic acid	Present study
TAFs	Walnut	99.00	15.00	HPLC	Citric acid	Present study
TAFs	Peanut	96.07	15.00	HPLC	Propionic acid	Present study
TAFs	Food	93.00	8.00	LC-MS/MS	Cold atmospheric pressure plasma (CAP)	(Hojnik, Modic et al. 2019)
TAFs	Hazelnuts	80–100	40.00	GC	Infrared rays and hot air	(Siciliano, Dal Bello et al. 2017)
TAFs	Wheat	81.00–95.00	30–180	HPLC and ELIZA	Ozone	(Savi, Piacentini et al. 2015)
AFM_1	Yogurt	78.63 ± 0.52	120.00	HPLC	*Lactobacillus rhamnosus* strain	(Zhang, Li et al. 2019)
AFB_1	Wheat	69.30	20.00	HPLC	8 KGy gamma rays	(Mohamed, El-Dine et al. 2015)
TAFs	Corn	57.00	120–480	HPLC-FD	Ozone exposure	(Porto, Trombete et al. 2019)

TAFs: Total aflatoxins, AFB_1: Aflatoxin B_1, AFM_1: Aflatoxin M_1.

List of Figures

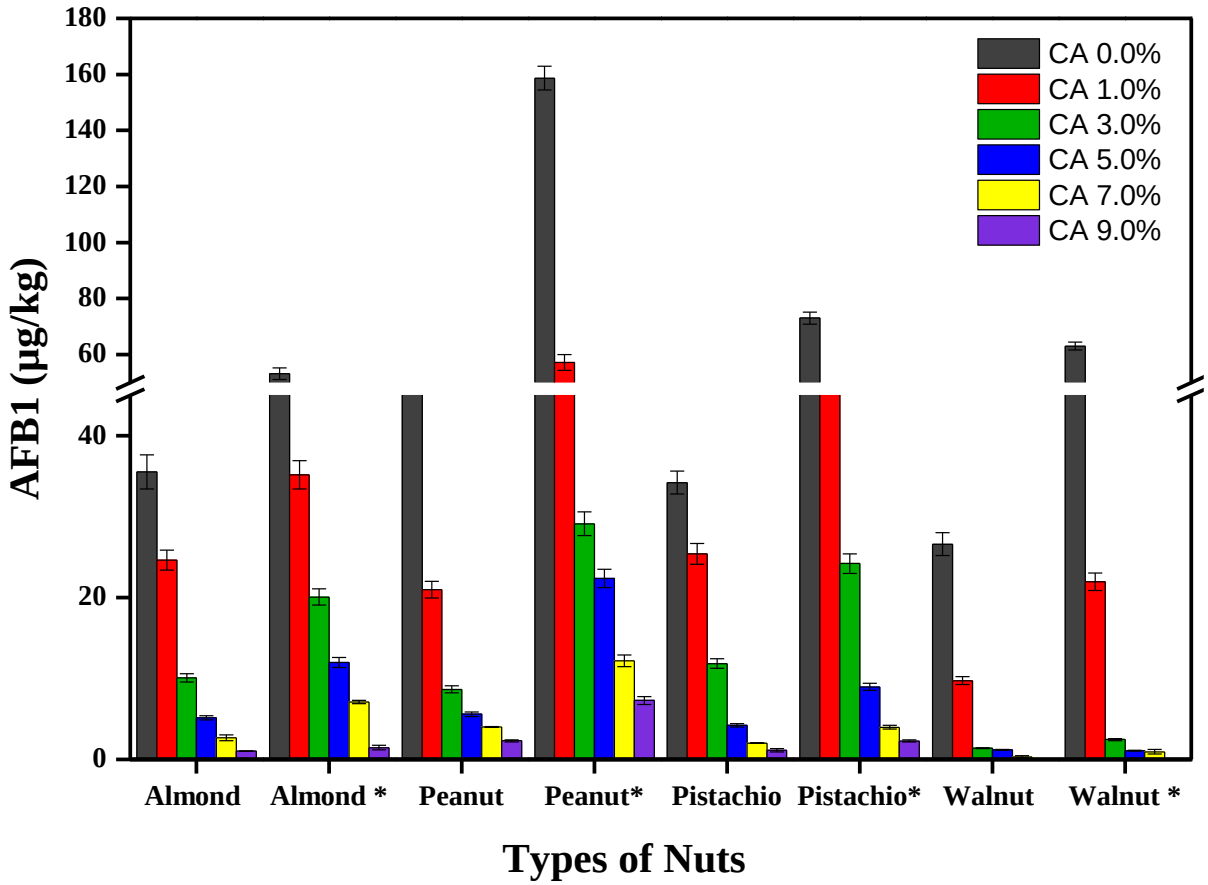


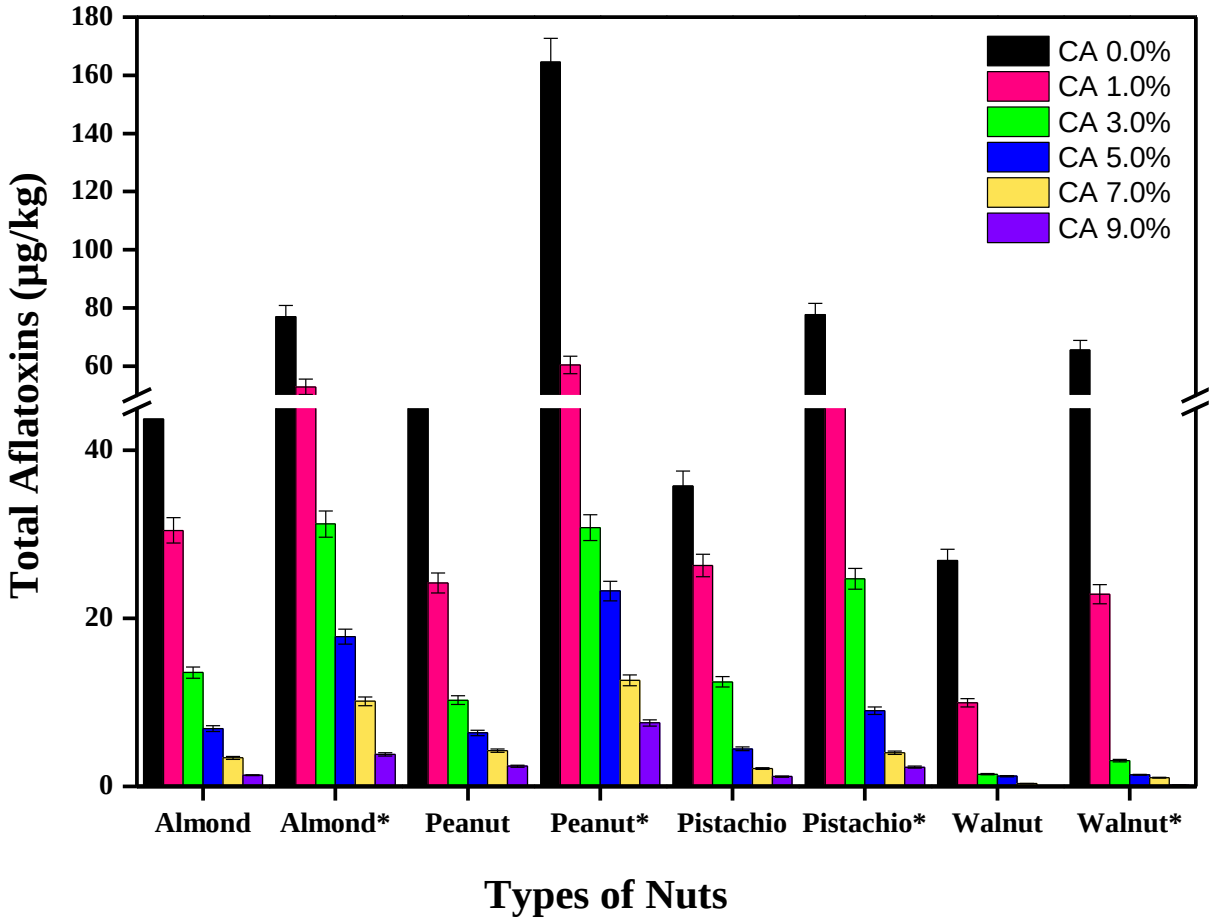
Fig. 1. Effect of different concentrations of citric acid (CA) on AFB_1 in-ground and tree nut at different moisture levels. Nuts marked with a star (*) are adjusted at 16±3% (high) and those without a star are adjusted at 10±3% (low) moisture levels.

638

639

640

641
642



643

644 **Fig. 2**. Effect of citric acid (CA) on total AFs content in-ground and tree nut at different moisture
645 levels. Nuts marked with a star (*) are adjusted at 16±3% (high) and those without a star are
646 adjusted at 10±3% (low) moisture levels.
647

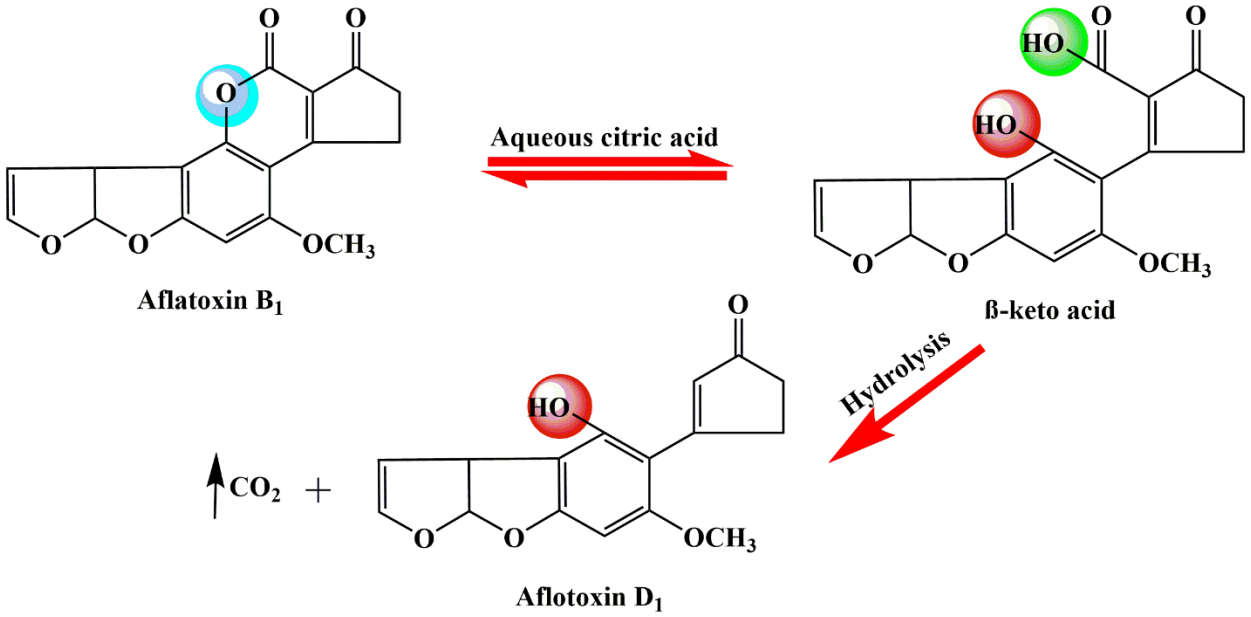


Fig. 3. The mechanism of detoxification of AFB_1 by citric acid.

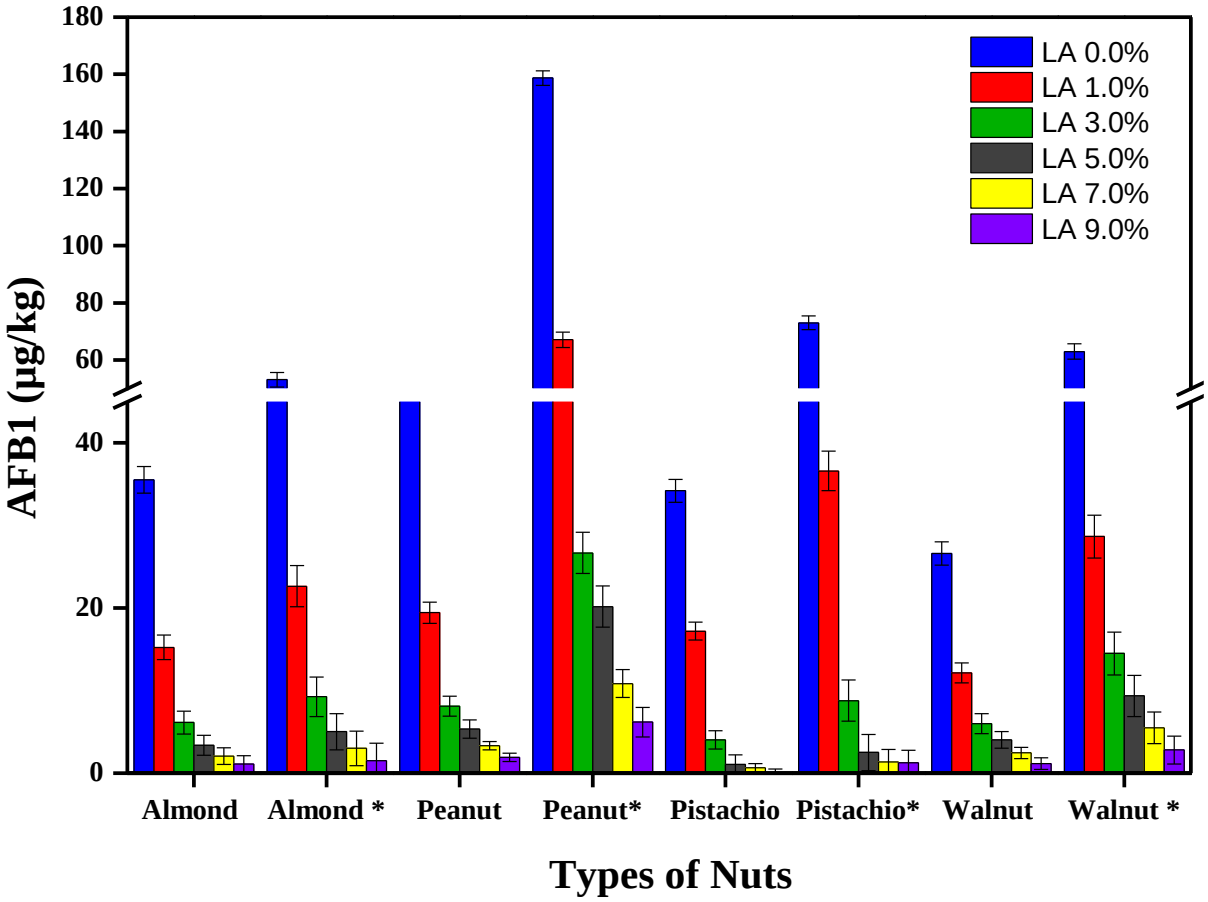


Fig. 4. Effect of different concentrations of lactic acid (LA) on AFB_1 in-ground and tree nuts at different moisture levels. Nuts marked with a star (*) are adjusted at 16±3% (high) and those without a star are adjusted at 10±3% (low) moisture levels.

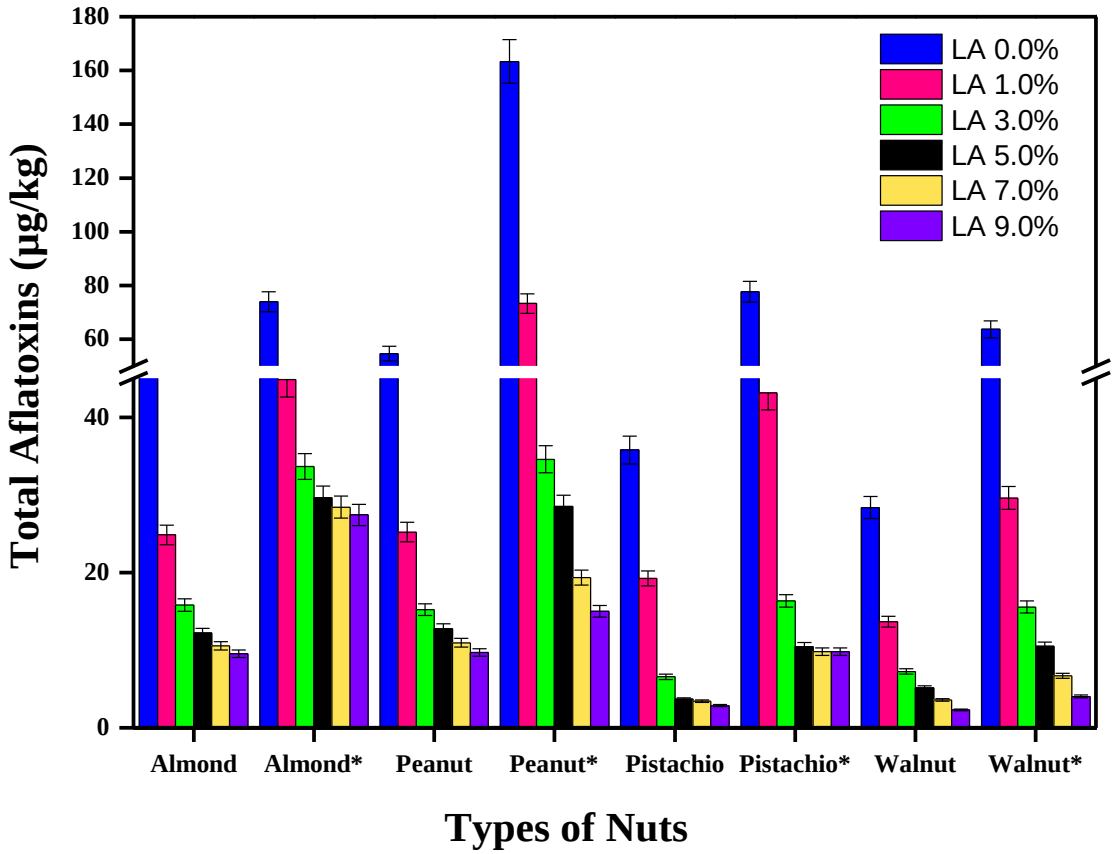


Fig. 5. Effect of different concentrations of lactic acid (LA) on AFB_1 in-ground and tree nut at different moisture levels. Nuts marked with a star (*) are adjusted at 16±3% (high) and those without a star are adjusted at 10±3% (low) moisture levels.

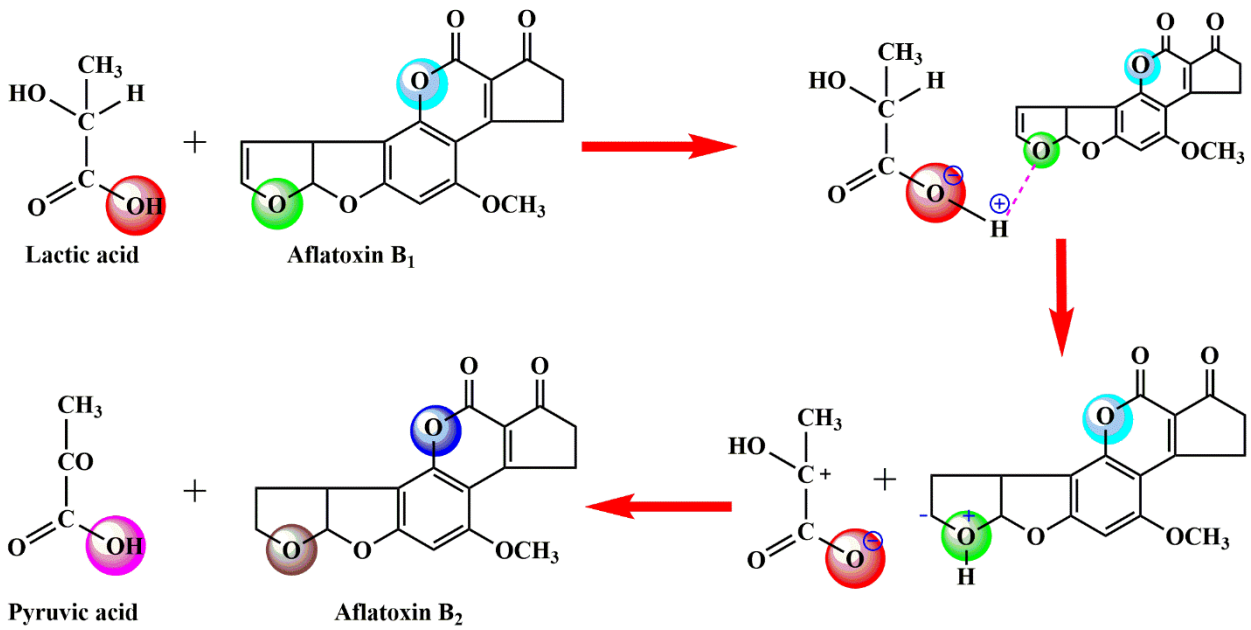


Fig. 6. The degradation mechanism of AFB_1 in nuts by lactic acid.

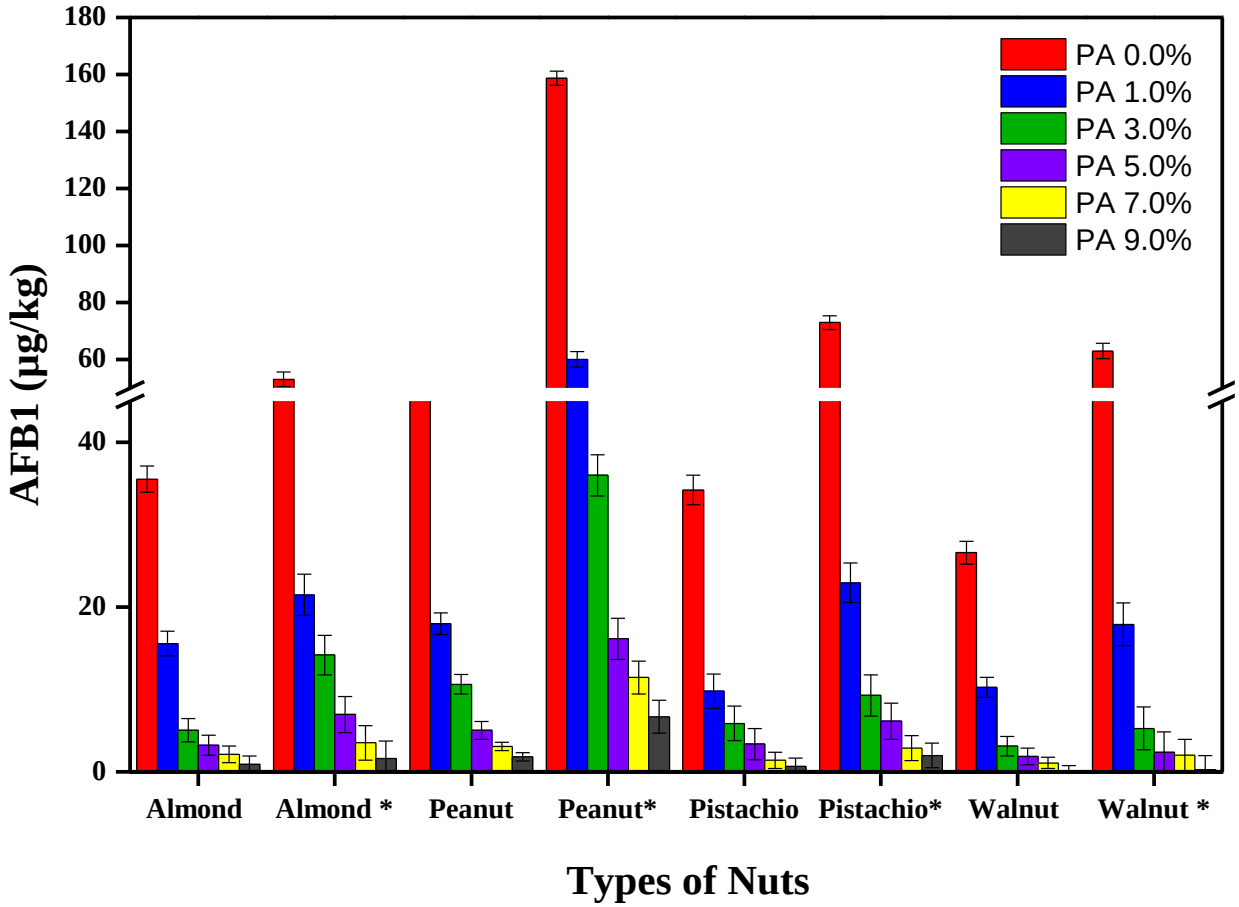


Fig. 7. Effect of different concentrations of propionic acid (PA) on AFB_1 in-ground and tree nuts at different moisture levels. Nuts marked with a star are adjusted at 16±3% (high) and those without a star are adjusted at 10±3% (low) moisture levels.

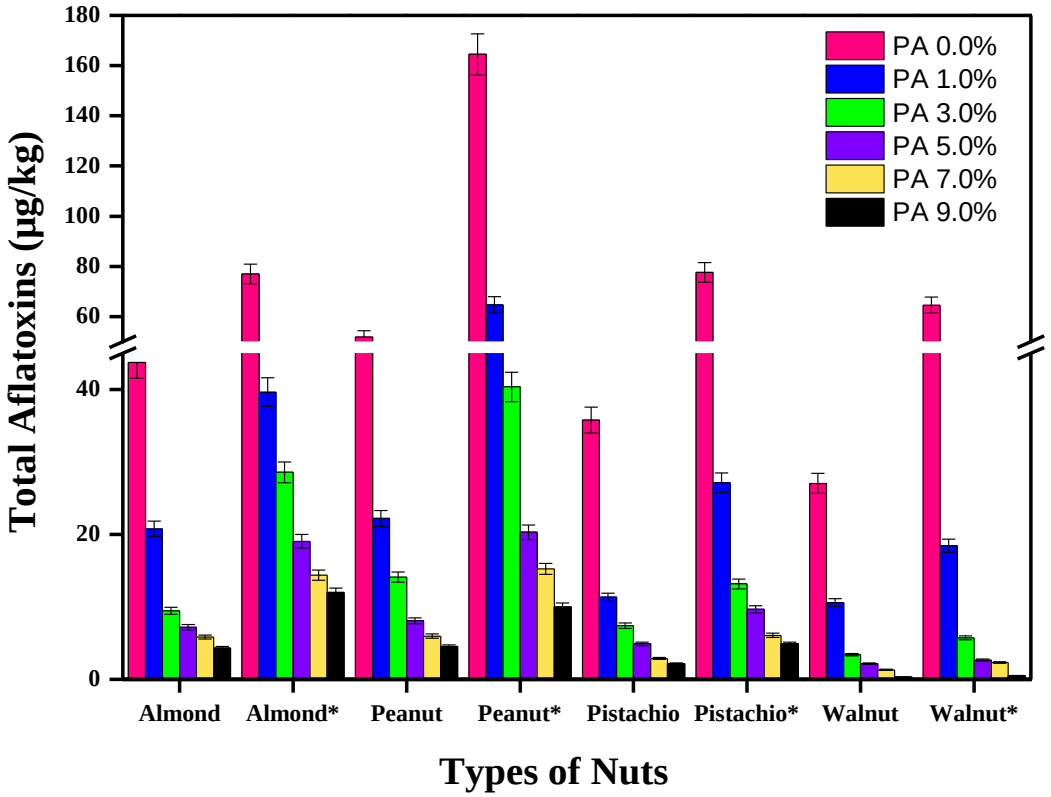


Fig. 8. Effect of different propionic acid (PA) concentrations on total AFs content in-ground and tree nut at different moisture levels. Nuts marked with a star (*) are adjusted at 16±3% (high) and those without a star are adjusted at 10±3% (low) moisture levels.