



Determination of the levels of aflatoxin contents in peanut and peanut products

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Abstract

Peanut are commonly consumed in all age groups and can be used directly roasting grain, chocolate coated, paste and candy. However, the products are easily exposure for different moulds growth and productions of aflatoxins. Therefore, the aim of this study was determination of aflatoxin contents in peanut and peanut product samples. For the present investigation, four raw peanut samples were collected from (Shebe, Gutin, Selamber and Bako Gazer) and six peanut product samples were collected from local markets. The aflatoxins were extracted using (AOAC, 2005) method with a mixture methanol and distilled water (80:20) as a solvent of choice for extraction. High performance liquid chromatography with fluorescence light detector (HPLC-FLD) was used for determination of aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) from samples. The result showed that the concentration of the most toxic strain (AFB₁) and the total concentration of aflatoxin for the four peanut samples were in the range from 0.18 to 65.67 µg/kg and 0.416 to 185.2 µg/kg respectively. Similarly, from the total six selected peanut product samples aflatoxin contamination were observed in the two locally produced peanut butter samples and concentration of (AFB₁) and total concentration of aflatoxin for the two local peanut butter samples (LPPBH and LPPBT) were (16.94 and 18.8) µg/kg and (49.202 and 41.273) µg/kg respectively. Overall, in the present investigations two of raw peanut and two of peanut product samples were contaminated with aflatoxin and the result was exceeded than permissible limits that recommended by United State, Food and Drug Administration and European Commission.

Keywords: gutin, bako gazer, shebe, aflatoxins, peanut

1. Introduction

The word mycotoxins come from the two Greek words of “Mykes” meaning moulds whereas “toxicum” meaning poisons [1]. Mycotoxins are a secondary metabolite of microscopic fungi which are not indispensable to the fungi life but shows toxic effects on humans and animals [2]. Particularly mycotoxins can appear in the food chain as a result of fungal infections of crops either by being eaten directly by human or used as livestock feed. Recent research finding report indicates that several kinds of mycotoxins are existed different part of the country especially, tropical and sub-tropical areas. For instance, around 300 different kinds of mycotoxins are known and which are produced about 200 different fungi species and primarily dominated tropical and sub-tropical areas [3]. Among them, Aflatoxins, Ochratoxins, Fumonisin, Trichothecenes, Deoxynivalen and Zearalenone (ZEA) are the major groups of mycotoxins and widely studied in the world [4]. However, from the above mentioned mycotoxins types currently the effects of aflatoxins are received the primary attentions in all over the worldwide associated with trade, economic, public health and food security sector. The reason, it leads adverse health effects for both animal and human being that consumes aflatoxins contaminated feeds and food stuffs [5]. But, the most toxic aflatoxins types are widely existed in different agricultural commodity commonly in a soybean, peanut, corn and different species [6]. Main factors for the developments of different fungi species particularly aflatoxins in agricultural commodities and different food stuffs due to different environmental factors. Primarily, temperature, humidity and high moisture contents are play a significant role for growth of moulds and contributed favorable conditions for the developments of aflatoxins on

commodities [7]. In addition, aflatoxins contamination was observed and occurred different food stuffs particularly peanut and peanut contains food stuffs through the three common factors such as, Physical, chemical and biological factors. In case of physical factors include temperature and moisture, chemical factors include the composition of the air and the nature of substrate whereas biological factors are those associated with the host species [8]. Moreover, agricultural commodities such as groundnut, maize, wheat, barley, oil seed and their products are susceptible to fungi attack either pre-harvesting or post harvesting factors. In case, of pre-harvesting factors such as due to the presence of chronic drought, heavy rains, crop insect damage, poor fertility; weed competition and high crop densities. Likewise, the crops products and different packed food stuffs can be exposure and inevitable for aflatoxins contamination during post harvesting conditions due to improperly drying of the products, high temperature and high moisture contents [9, 10, and 11]. But the most relevant food items that have been reported to aflatoxins are cereal grains (maize, rice, wheat, and sorghum), oil seed (groundnut, cotton seed, coconut, soybean, and sunflower) and Vegetable oils such as peanut oil, coconut oil and cottonseed oils are widely discussed by several researchers and confirmed the presence aflatoxins contents in those mentioned crops products [12, 13]. Aflatoxin contaminations of food cause hepatotoxicity, carcinogenicity, mutagenic and impaired central nervous system. Not only for public health hazards, but also leads negative impacts on society and international economy through spoilage of the commodity [14, 15].

Therefore, due to the lack of well documented information about the effects of aflatoxins in peanut and peanut

contained food stuffs in Ethiopia and lack of awareness towards on aflatoxins causes in different part of the country. However, few papers have been conducted by different researchers in different part of the country. For instance, a research was conducted in Brazil 240 peanut product samples was collected from four sample areas (Araras, Leme, Pirassununga and Porto Ferreira) were collected from June 2006 to May 2007. The result indicate that 9 samples contained high concentrations level of aflatoxins and exceeded than the permissible levels where as 106 samples contained from 5.6 – 18.89 µg/ kg range of aflatoxins concentrations ^[16].

Likely, a research was conducted on the occurrence of aflatoxin contaminations in peanut and peanut based products were done in India on a total of 27 samples were analyzed with High Performance Liquid Chromatography (HPLC-FLD). The result indicate that 21 peanut samples(77.7%) were below limit of detections(LOD) and the two peanut product samples (peanut sack and roasting) contains 16.3 µg/kg and 8 µg/kg of aflatoxins concentration respectively ^[17]. However, there is no such kind of report on Ethiopia about the raw peanut and peanut product. It has to be noted that the products have highly cultivated and majority of the society are depends their own life on this commodities. In addition, plant species, geographical locations, and overall climatic conditions of the region, harvesting season and storage conditions of the products are vary. Thus, the aim of this study was to determine the levels of aflatoxins contents from peanut and peanut product in Ethiopia.

2. Materials and Methods

2.1 Chemical and Reagents

All the chemicals and reagents used in this study were of analytical grade reagents with highest Purity. These chemicals and reagents were: Methanol (Research Lab Fine Chem. Industries, Mumbai, India); Distilled water for extraction of aflatoxins; n-hexane(Research-Lab Fine Chem. Industries, Mumbai, India) for defatting; Sodium chloride(BDH Laboratories Supplies, Poole, England) for absorption of moisture contents; Disodium phosphate hydrate(BDH Laboratories Supplies, Poole, England); Sodium phosphate hydrate (BDH Laboratories Supplies, Poole, England)and Tween® (Research Lab Fine Chem. Industries, Mumbai, India) for clean up

2.2 Apparatus and Equipments

Apparatus and instrument used during the experiments were Mortar, Pestle, Poly ethylene bag, Aluminum foil, Electronic balance, Spatula, Oven, Crucible, Immunoaffinity column(IAC) clean up, Whatman filter paper, Lab stands with a clump, Vacuum pump, Volumetric flask, Micro pipettes, measuring cylinder, Beakers, Conical flasks, Sample collecting bottle, Orbital shaker, Syringe, Vials with screw cap, separatory funnel and Racker and Agilent Technologies HPLC system set up contains auto sampler, Injector, Column, Dervatizer, Degasser, Fluorescence light detector and Desktop computer with open lab EZchrome software.

2.3 Description of Study area

The study was conducted in Oromia and Southern, Nation, Nationalities and People of Regional State of Ethiopia. Bako

Gazer, Selamber, Gutin and Shebe were selected in this study from two Regions. The sampling areas were chosen based on their commercial importance and for their high production capacity of peanut product. In addition, products are widely consumptions by society. Geographical location of the study areas are described under Table 1below.

Table 1: Geographical location of study areas

Sample area	Latitudes	Longitudes
Selamber	6°29'0"	37°28' 0 "
Bako-Gazer	5°54'30 "	36°35' 0 "
Gutin	7°31' 0 "	36°31' 30 "
Shebe	9°35' 0 "	36°38' 30 "

2.4 Sample collection and sample preparations

Raw peanut samples were collected from the two different Ethiopian regions (Oromia and Southern, Nation, Nationality and People). Sixteen raw peanut sample (four peanut sample from one study area) were collected each sampling site to prepared bulk samples. Accordingly, a total of four bulk samples, one from each study area. Similarly, peanut product samples were collected from the three local markets. Locally, produced peanut butter samples were collected from Dilla town supermarket (three supermarket was randomly selected and from each supermarket two different locally produced peanut butter samples were collected. The same brands of sample was homogenized and reduced into two samples. Likewise, imported peanut butter samples were collected from Hawassa supermarkets and like that Dilla town, six imported peanut butter samples were collected from this town and homogenized into two samples. Whereas, chocolate coated peanut samples were collected from Addis Ababa (from five different supermarkets ten samples were collected and homogenized into two samples based on their brand. Finally, the purchased samples were placed in polyethylene bag and transported to laboratory for further analysis.

2.5 Determination of Moisture

Moisture were determined according to Association Official of Analytical Chemist (AOAC, 2000) using the official method 925.09. A crucible were dried in an oven at 105°C for 1 hour and placed in desiccators to cool. The weight of the crucible (W₁) was determined. 5g samples were be weighed in the dry crucible (W₂) and dried at 105°C for 3 hours and after cooling to room temperature in desiccators it was reweighed (W₃). The moisture content were determined as (Eq.1)

$$\text{Moisture contents \%} = \frac{W_2 - W_3}{W_2 - W_1} * 100 \dots\dots\dots \text{Eq (1)}$$

2.5.1. Extraction of aflatoxin from peanut and peanut product sample

Extractions of aflatoxin from test samples were taken place according to the official methods of Association Organization of Analytical Chemists (AOAC, 2005.08) ^[18]. From the homogenized and well mixed of peanut and peanut product samples 20 g of sample and 2 g of sodium Chloride (NaCl) were weighted by analytical balance with 50 mL of n-hexane transferred in to 250 mL of conical flasks. Then mixed with 100 mL of methanol and distilled water (80:20 v/v) proportion and the mixture were shaken at 640 rpm for 50 minutes in an orbital shaker. Then extracted samples

were filtered and the filtrate samples were transferred into a separatory funnel. After filtration, seven milliliters (7 mL) of the extracted sample was added to forty-three milliliters (43 mL) of phosphate buffer saline (PBS) at pH 7.2 in sample to buffer solution proportions becomes 7:43(v/v) by volume. Then an aliquot fifty milliliters (50 mL) was passed through AflaTest® Immunoaffinity column (IAC) at flow rate of one to two drops per second. Finally, the column was with 2 mL of pure high performance liquid chromatography graded methanol was eluted and the eluate was collected in an amber vial. Finally, 20 µL were injected into reversed phase high performance liquid chromatography (RP-HPLC) with fluorescence light detector.

2.6 Statistical analysis

A triplicate measurement of each sample was carried out and the data was obtained from the chromatogram. One-way ANOVA and independent sample t-test using IBM SPSS Statistics 20 was used to verify whether there are significant differences in the data obtained from different sample study areas and peanut product samples. Differences were considered significant when $\alpha < 0.05$.

3. Result and Discussion

3.1 Moisture contents of Raw Peanut samples

The moisture contents of the substrate and temperature are the main factors regulating the fungal growth and favorable conditions for the formation of aflatoxins in grain. For instance, a moisture content of 18% for starch cereal grains and 8–10% for oil-rich nuts and seeds has been established for maximum production of the toxin [19]. According to Codex Alimentarius Commission, the maximum allowable range can be supported for different mould developments and productions of aflatoxins on raw peanuts occurred when the moisture contents ranged from 8 to 10% [20]. Therefore, the moisture contents of the four selected raw peanut samples are:

Present investigations result was described under Table 2 below

Table 2: Moisture contents of raw peanut samples

Study area	Moisture % (mean ± SD)
Shebe	5.72 ± 0.11
Gutin	6.74 ± 0.16
Selamber	5.69 ± 0.09
Bako Gazer	8.58 ± 0.18

The results of moisture contents of the four selected raw peanut samples are given in Table 2. Among the four selected study areas, the highest percentage of moisture contents was recorded in two peanut samples (Gutin and Bako Gazer) and the lowest level (5.69 and 5.72) percentage was observed in Selamber and Shebe peanut sample respectively. According to Codex Alimentarius Commission standards, in the present study only one study area sample (Bako-Gazer) is above the maximum allowable limits and the remainder study areas are below the limit of the standards. However, it was observed that present findings with the previously reported data, in the current study relatively higher moisture contents were observed in peanut samples. For instance, in the current finding the moisture contents of the four selected peanut samples ranged from 5.69–8.58% it is higher than 3–6.8% in the previously reported data [21].

3.2 Chromatogram for raw peanut sample

Before directly injecting the extracted sample into the instrument (High performance liquid chromatography-Fluorescence light detector) the prepared standard solution was injected and obtained the chromatogram. Following that extracted raw peanut samples were injected into the instruments (HPLC-FLD) and the obtained chromatogram for each study area of raw peanut samples was described in Fig. 2, Fig. 3 and Fig. 4 respectively.

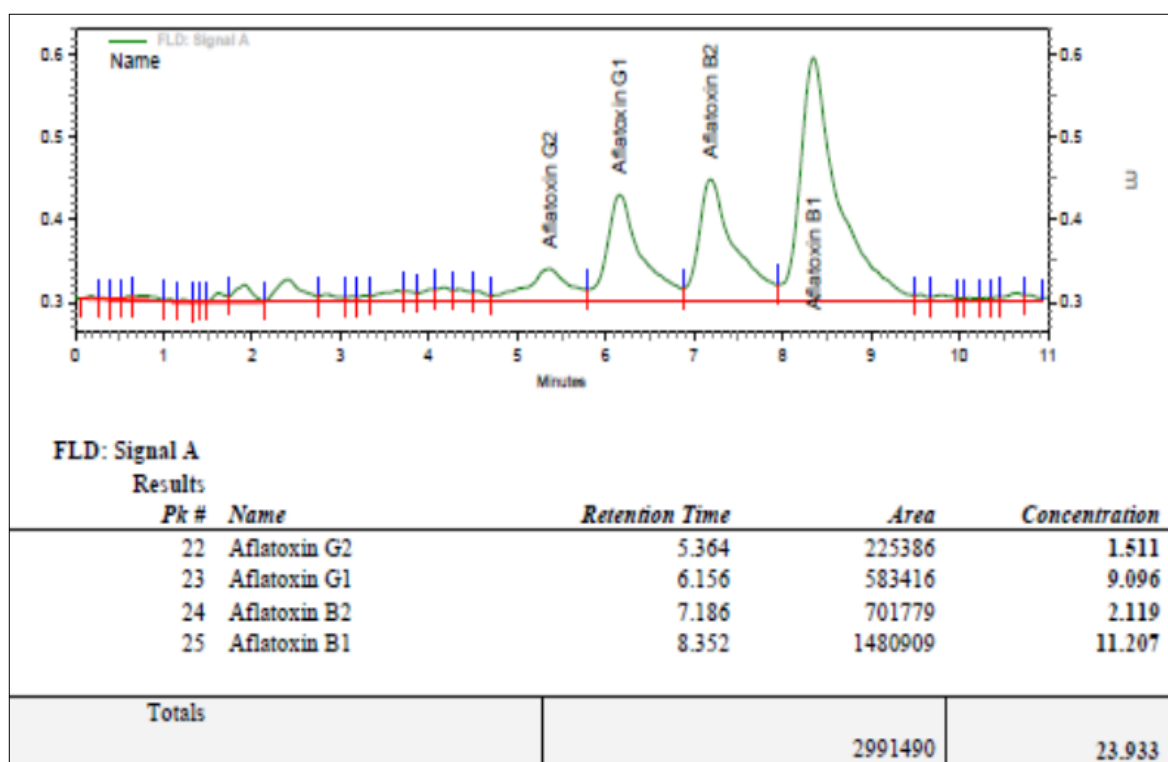


Fig 1: Chromatogram for standard solutions

In the present study only the two study areas raw peanut samples (Gutin and Bako-Gazer) are highly contaminated with aflatoxins and chromatogram of the samples were described below in Figure 2 and Figure 3 respectively. As the chromatogram report indicate that well resolved peaks for aflatoxin B₁, B₂, G₁ and G₂ was observed in Gutin study

areas raw peanut sample. But in case of Bako-Gazer study areas sample the peak separation was observed only the two aflatoxin types AFB₂ and AFB₁ and the remainder aflatoxin types (aflatoxin G₂ and aflatoxin G₁) is not clearly separated from back ground noise level and detected below the limit of detection.

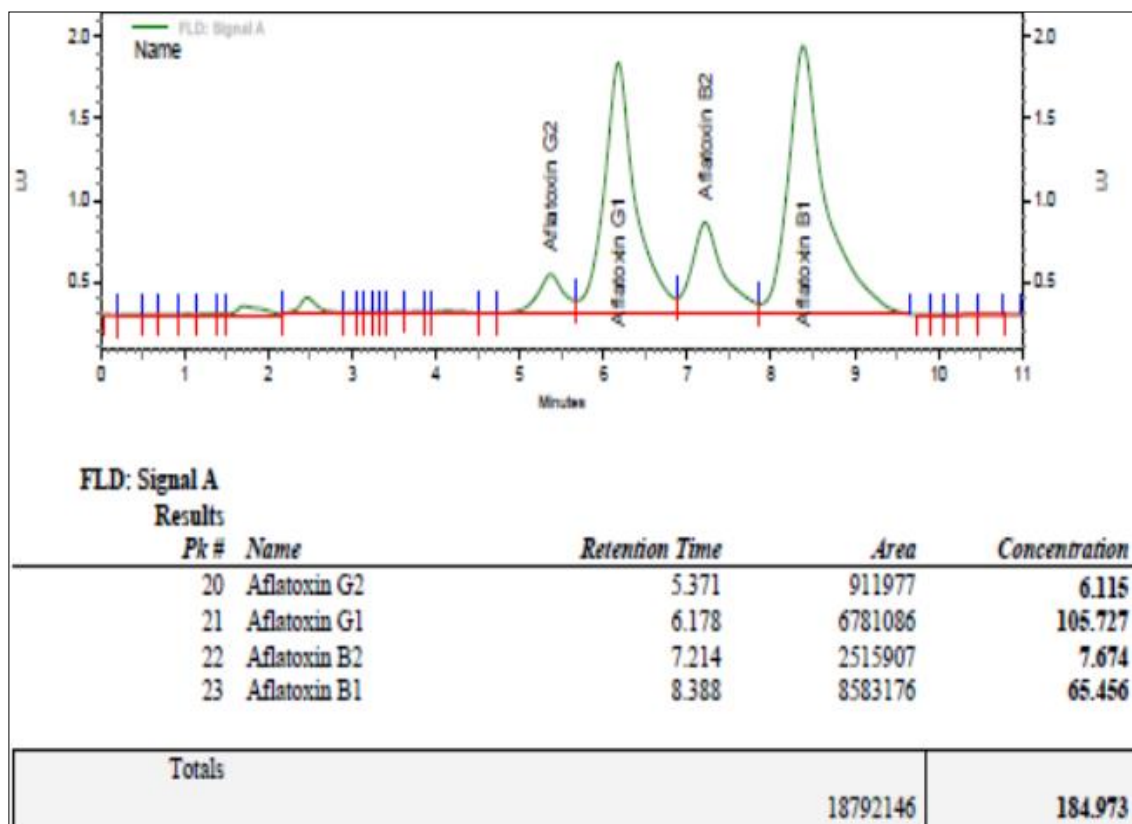


Fig 2: Chromatogram for Gutin raw peanut sample

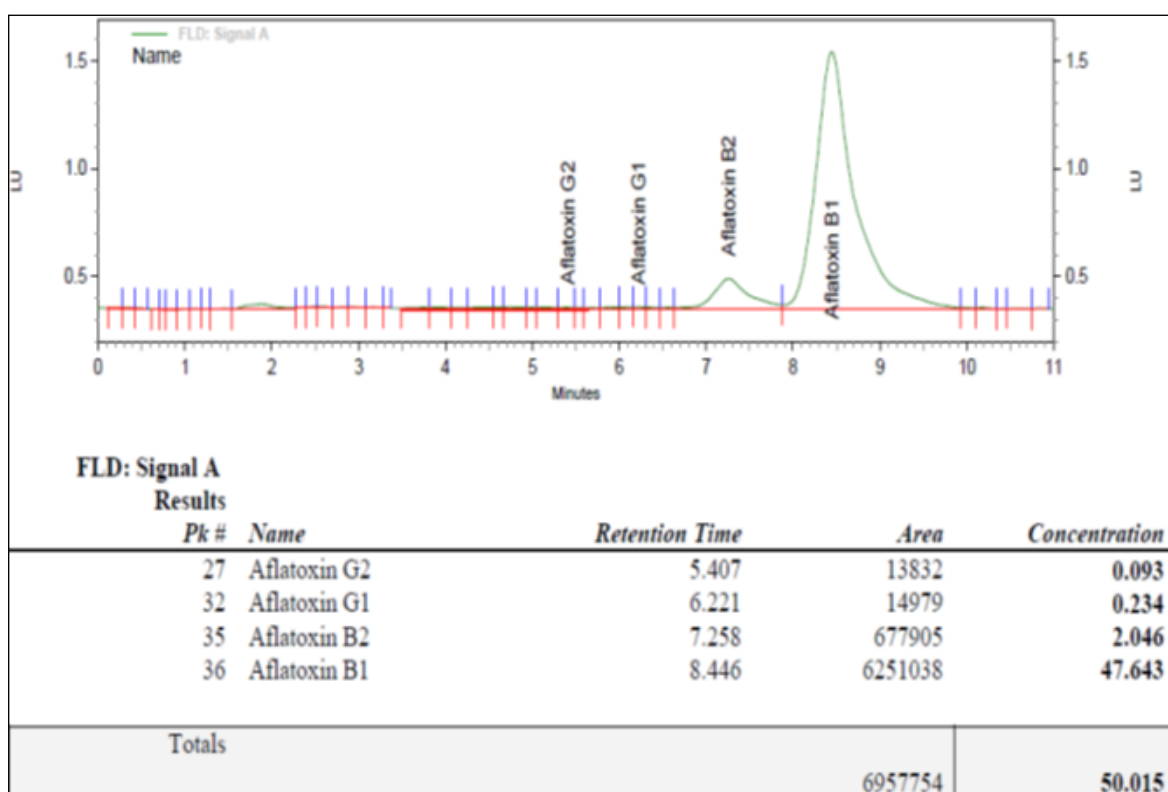


Fig 2: Chromatogram for Bako-Gazer raw peanut sample

3.2.1 Aflatoxin Levels in Raw Peanut sample

In the present study from the four selected raw peanut samples, the two study areas (Gutin and Bako-Gazer) raw peanut samples are highly contaminated and its concentrations levels are exceeded than the permissible limits that recommended by US. Food and Drug Administrations. The result of aflatoxins levels that detected in samples are described in Table 3 below.

Table 3: Aflatoxin level in raw peanut samples

Study area	Aflatoxin level in peanut samples ($\mu\text{g}/\text{kg}$)				
	AFG ₂	AFG ₁	AFB ₂	AFB ₁	Total
Shebe	0.032	0.178	0.025	0.181	0.416
Gutin	6.115	105.73	7.674	65.67	185.19
Selamber	0.091	0.503	0.037	0.268	0.899
Bako Gazer	0.093	0.234	2.234	47.64	50.204

From the above Table 3 result, Gutin peanut sample was more contaminated than the remainder of the three study sites (Selamber, Bako Gazer and Shebe). This highest level of aflatoxin contamination was observed in this study area might be due to environmental factors. The data that gathered from National and Meteorology Agency the average temperature and humidity of this study area 25.28 °C and 69.54% respectively. So, such factor may contribute the sample to exposure for aflatoxins contaminations. Because, temperature and relative humidity are the crucial factors for accumulations of aflatoxins in food and food staffs [22]. In addition, the present investigations are agreements with the previous studies. For instance, in previous report indicate that in peanut and animal feeds are easily exposure for the developments of moulds and formations of aflatoxins when the temperature ranged above 25°C and humidity level become greater than 62% [23].

Likewise, Bako Gazer peanut sample was the second highest level of aflatoxins contaminations compared with the remainder of two study site (Shebe and Selamber) but lower than Gutin raw

peanut sample. In Bako Gazer peanut sample exposure for aflatoxin might be the influence of high percentage of moisture contents. Because, the previous report indicate that fungi species particularly *A.flavus* growth and cause for aflatoxins formations are correlated with moisture contents of the raw peanut ranged from 8 – 10% [24]. Therefore, among the four selected study areas, high moisture contents were recorded in Bako Gazer study area peanut samples and this might be contribute the sample exposure for aflatoxins contaminations.

On the other hand, in this two study areas peanut sample the result that observed highly exceeded than the maximum allowable limit that recommended by different International organizations. For example, United State Food and Drug

Administration have set the limit for aflatoxin B₁ (AFB₁) and total aflatoxin at 10 and 20 $\mu\text{g}/\text{kg}$ and European Commission established the current limit for AFB₁ and total aflatoxin 2 and 4 $\mu\text{g}/\text{kg}$ respectively for peanut, dried fruit and proceeds foodstuffs [24].

However, the result that observed in the present study is very low compared with the pervious reported data. For instance, a research was conducted in Brazil in 1991, one thousand forty -four (1044) samples was collected and analyzed for an aflatoxin, and nine hundred forty (940) samples are forms positive with an aflatoxins and concentration were ranged from 30 – 5000 $\mu\text{g}/\text{kg}$ [104]. Likely, a survey was conducted in Brazil in 1999, one hundred and thirty seven (137) sample was collected and analyzed by Thin Layer of Chromatography (TLC), 45% of the samples were positive for an a aflatoxin and the concentration were ranged from 5 to 382 $\mu\text{g}/\text{kg}$ [7]. The result from both study indicated that, the samples were highly contaminated and results are contradicted with the present finding results. In case of current finding the total concentration of aflatoxin level was recorded in the range 0.416 – 185.19 $\mu\text{g}/\text{kg}$. Moreover, research was conducted in Malaysia in 2010 on five peanut samples the incidence of aflatoxin B₁ (AFB₁) were detected in the ranged from 0.2 – 101.8 $\mu\text{g}/\text{kg}$. Likewise, research was conducted on determination of aflatoxin level from stored peanut samples in Sudan in 2010. The concentration of aflatoxin B₁ (AFB₁) was observed from the range of (17.57 – 400) $\mu\text{g}/\text{kg}$ [25, 26]. It is also very high when it is compared with the present study of the research finding and the concentration ranges that detected in four peanut samples were 0.181 – 28.44 $\mu\text{g}/\text{kg}$. On the other hand, ANOVA result showed that there was a significant difference between the aflatoxin level that recorded in each study site

3.3 Chromatogram for peanut product sample

Similarly to that of raw peanut sample, from the six selected peanut product samples aflatoxin contamination was observed only the two locally produced peanut butter samples. Then chromatogram for those contaminated peanut product sample was described in Figure4 and Figure5 respectively. In case of LPPBH sample the peak corresponding to AFG₂, AFG₁, AFB₂ and AFB₁ were clearly separated from the back ground of noise level and retention times of the individual aflatoxins (AFG₂, AFG₁, AFB₂ and AFB₁) were approximately 5.36 min, 6.17 min, 7.20 min and 8.37 min respectively. Similarly, LPBT that peak corresponding to AFG₂, AFG₁, AFB₂ and AFB₁ were clearly separated from back ground noise level and the retention time of the individual aflatoxin (AFG₂, AFG₁, AFB₂ and AFB₁) in this peanut butter samples were approximately 5.35 min, 6.15 min, 7.17 min and 8.34 min respectively.

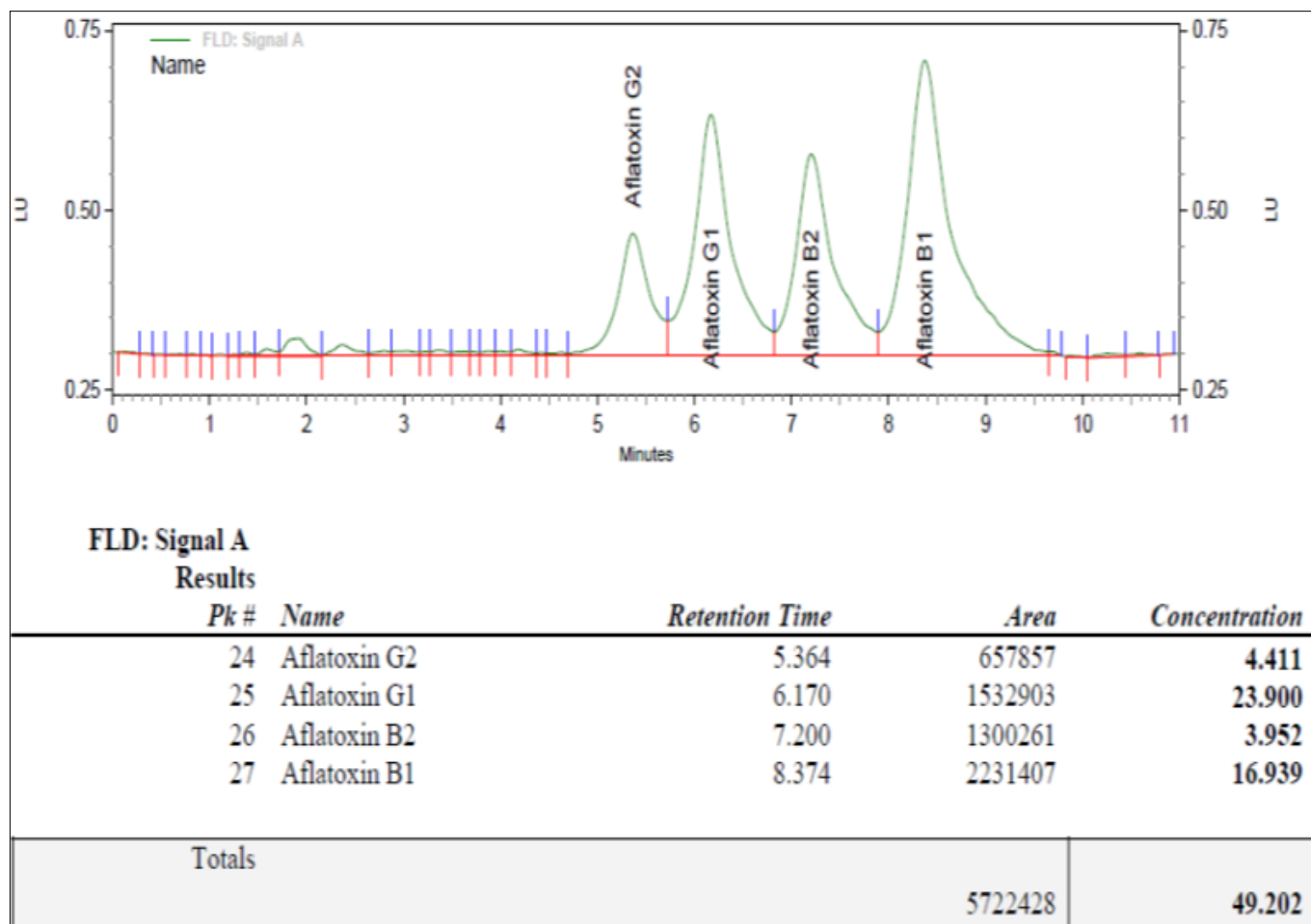


Fig 4: Chromatogram for LPPBH butter

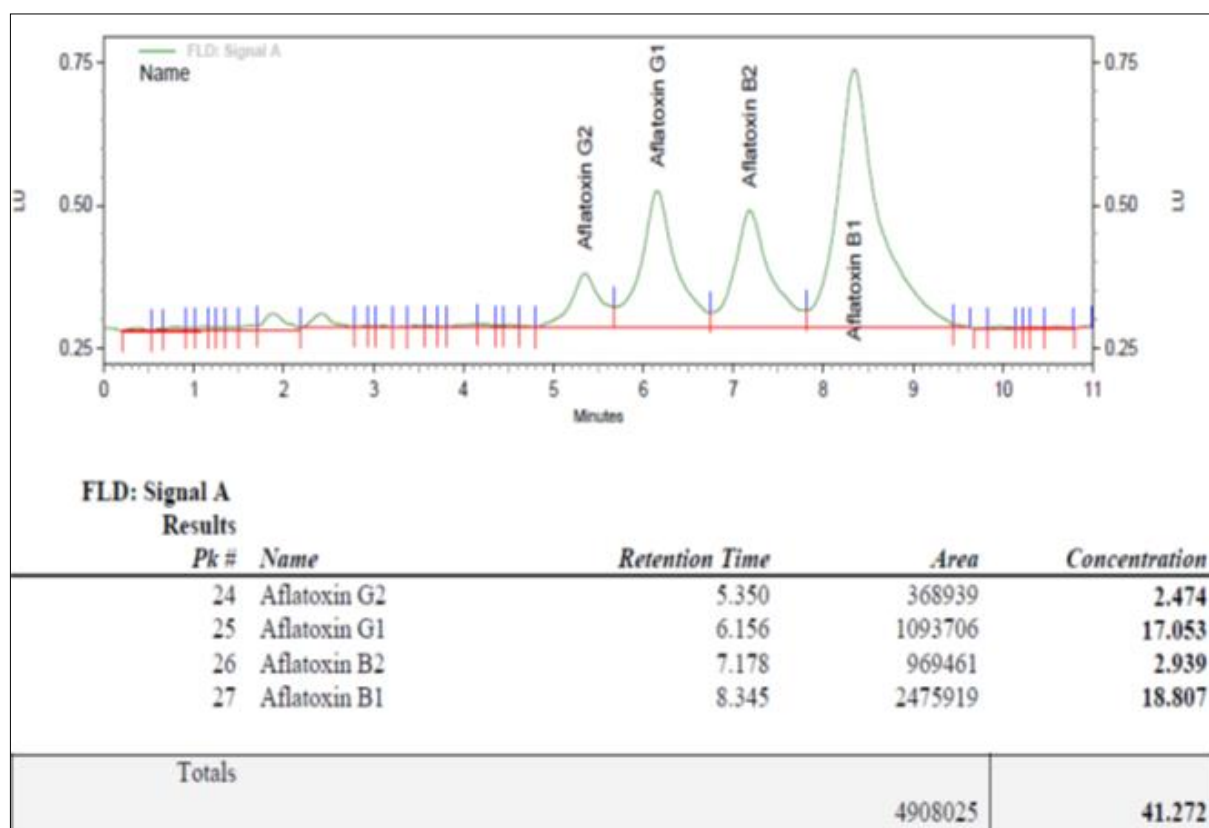


Fig 5: Chromatogram for LPPBT butter

3.3.1 Aflatoxin levels in Peanut Product samples

High level of aflatoxin contaminations are great concern on human health. Especially due to the fact that peanut and peanut contained food stuffs are largely consumed by children and uniquely susceptible for the effects of aflatoxin effects. So, the purpose of this study to determine the levels of aflatoxins contents from peanut contain of food stuffs. Therefore, six selected peanut product samples were collected from local markets and the results of each peanut product samples were described in the (Table 4) below

Table 4: Levels of aflatoxin in peanut product samples

Aflatoxin level in ($\mu\text{g}/\text{kg}$)					
Sample type	G ₂	G ₁	B ₂	B ₁	Total
Chocolate coated peanut (Brazil)	0.074	0.167	0.024	0	0.265
chocolate coated peanut (India)	0.065	0.153	0.035	0.025	0.278
LPPBH	4.411	23.9	3.952	16.939	49.202
LPPBT	2.474	17.053	2.939	18.807	41.273
America brand peanut butter	0.063	0.133	0.192	1.431	1.819
India brand peanut butter	0.041	0.157	0.052	0.569	0.819

LPPBH = locally produced peanut butter sample collected from "H" company, LPPBT= locally produced peanut butter sample collected from "T" company

As Table 4 showed results indicate that the two locally produced peanut butter sample that collected from (H and T) company samples were highly contaminated with aflatoxins. But when compared the aflatoxin level that recorded in this two locally produced peanut butter highest level of aflatoxin B₁ and aflatoxin G₁ was recorded in T company peanut butter sample and the concentrations were (18.807 and 17.053) $\mu\text{g}/\text{kg}$ respectively. However, the result that recorded both locally produced peanut butter samples are highly exceeded than the maximum allowable limits that recommended by both US, Food and Drug Administrations and European Commissions. It was concluded that the reason for the highest level of aflatoxin contamination was observed in locally produced peanut butter sample compared with imported peanut product might be insufficient control of transport, the way of handling of the producer company during processing and storage conditions of the supermarket may contributed the products for exposure of aflatoxin contaminations. For instance, one of the documented research finding report indicate that poor storage conditions and infrastructure are their own a significant role for aflatoxins contaminations and the effects of mycotoxins are high in developing countries [27]. Hence, this two locally produced peanut butter products are not safe for human consumption, international trade and for afro-processing based on the recorded levels of aflatoxins. On the other hand, comparing of the current finding with the previous reported data, the result that recorded in the present study is very low. For instance, the total concentration of aflatoxin that recorded in this selected six peanut product samples ranged from (0.265 – 49.2) $\mu\text{g}/\text{kg}$ generally very low compared with previous reported in 2013 in Kenya, in 1998 in Campinas of Brazil and concentration ranged (ND – 2377.1 $\mu\text{g}/\text{kg}$) and (43 – 1099 $\mu\text{g}/\text{kg}$) respectively [105, 106]. In addition, a research was conducted in Turkey in 2006 on determinations of an aflatoxins level from peanut butter and twenty peanut butter samples was analyzed by HPLC- FLD, all the samples are contaminated with aflatoxins and the level of aflatoxins ranged from 8.16 – 75.74 $\mu\text{g}/\text{kg}$ [28]. But in case of present investigation was in contrast with the

above mentioned results and the concentration range in six selected peanut product samples were (0.265 – 49.2) $\mu\text{g}/\text{kg}$.

3.4 Comparison of Aflatoxin level in Peanut sample in study site

The occurrence and levels of aflatoxin B₁ (AFB₁) for individual peanut butter samples (H Company, T Company) for local produced peanut butter and (India and America) for imported peanut butter samples the result was described under Table 5 below. As mentioned early, aflatoxins contamination was observed in locally produced peanut butter samples. In case of imported peanut product sample level of aflatoxins concentrations are below the limit of detections. The reason for lowest level of aflatoxin concentration was observed might be that developed countries have strictly control on the quality of food which reduces the chance for aflatoxin contaminations. For example, research data showed that most of the developed countries such as United State of America and some of European country the government great effort to controlling aflatoxin level from peanut and peanut contained food stuffs [29]. In addition, majority of the imported brands are usually their own quality control managements for each and every product in marketing systems [30].

Table 5: Aflatoxin level in local and imported peanut butter sample

Sample type	Aflatoxin level in (ppb)				
	AFG2	AFG1	AFB2	AFB1	Total
LPPBH	4.41	23.9	3.95	16.94	49.2
LPPBT	2.47	17.05	2.94	18.8	41.26
America brand PB	0.06	0.13	0.19	1.43	1.82
India brand PB	0.04	0.16	0.05	0.57	0.82

On the other hand, the recorded result was compared by using of independent sample t-test. The independent sample t-test statistically analysis result indicate that there is a significant difference between locally produced and imported peanut butter sample at 95% level.

4. Conclusions

In the present study, one can concluded that from a total of ten selected peanut and peanut product sample were analyzed and two of four raw peanut and two of six peanut product samples were exceeded the tolerable limits of both aflatoxin B₁ and total aflatoxin that recommended limit (10 and 20) $\mu\text{g}/\text{kg}$ of Food and Drug Administration. Hence, this two raw peanut and two locally produced peanut butter samples are not safe for direct human consumptions as per United State of Food and Drug Administration standard. However, out of the total selected samples two of four raw peanut and four of six peanut product samples were below the limit of detections and safe for consumptions purpose. Therefore, further investigation on locally produced peanut butter and two raw peanut samples those collected from Gutin and Bako-Gazer study areas should be carried out with a much larger sample size to confirm this result.

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