Yaman et al., J Food Nutr Disor 2019, 8:1
DOI: 10.4172/2324-9323.1000256

Journal of Food and Nutritional Disorders

Research Article

A SCITECHNOL JOURNAL

Determination of Aflatoxin Contents of Some Chips and Snacks with Peanut by Post-Column UV Derivatization System

Mustafa Yaman*, Jale Çatak, Halime Uğur, Elif Okur, Gülser Reyyan Çetinkaya, Seher Erdoğan, Serra Orak and Zehra Sağlık

Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin aim University, Istanbul, Turkey

***Corresponding author:** Yaman M, Çatak J, Department of Nutrition and Dietetics, Faculty of Health Sciences, İstanbul Sabahattin aim University, Istanbul, Turkey, Tel: +90 212 692 8915; E-mail: mustafa.yaman@izu.edu.tr

Received Date: September 04, 2018; **Accepted Date:** January 07, 2019; **Published Date:** January 14, 2019

Abstract

Aflatoxins are mycotoxins, mainly produced by *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nominus*. Mycotoxin is found in foods which stored in hot, humid and unhealthy conditions such as cereals especially maize and rice, some animal source foods such as milk and cheese, hard-shelled fruits such as walnuts, hazelnuts and peanuts, dried fruits and spices. Aflatoxins are composed of six main groups namely B_1, B_2, G_1, G_2, M_1 and M_2. There is a strong statistical relationship between aflatoxin uptake and primary liver cancer. The aim of this study is to determine the amount of aflatoxin B_1, B_2, G_1, G_2 in some chips and snacks with peanut consumed in Istanbul. In this study 10 different types of chips and 4 different types of peanut bars samples obtained from the markets in Istanbul were grinded and aflatoxin determination was performed. The analysis was carried out by HPLC using immunoaffinity chromatography and post-column UV derivatization system. B_1 and B_2 aflatoxins are determined in 50% of the peanut-chips in the range of 5-24 µg/kg. Aflatoxin B_1 values of 30% of peanut-chips found above the limit. G_1 aflatoxin was not found in samples. According to the related notification in Turkish Food Codex, it is determined that total aflatoxin amount is above the limit in 30% of chips products. The amount of aflatoxin determined in snack bars is below the maximum limit. According to the European Community, limit values for aflatoxin B_1 that can be found in human food is 2-4 µg/kg. In our country, the maximum acceptable value for peanuts, other oil seeds and spices is 5 µg/kg for aflatoxin B_1 and 10 µg/kg for total aflatoxin.

Keywords: Aflatoxin; Chips; HPLC; Peanut-snacks; Peanut

Introduction

Cancer, which is responsible for approximately 6 deaths globally, is the second most important cause of death worldwide. According to WHO data, 8.8 million people lost their lives due to cancer in 2015 [1]. According to the Turkey Statistical Institute data; cancer accounted for about 20% of all deaths in 2015 in our country [2]. The second most common cause of cancer deaths worldwide is liver cancer. Genetic factors as well as physical, chemical and biological carcinogens play an important role in cancer formation [1]. It is thought that 4.6% to 28.2% of hepatocellular carcinoma cases, the most common form of liver cancer, are caused by aflatoxin exposure from the chemical carcinogen class [3]. It has been proven in studies that there is also a strong statistical relationship between aflatoxin uptake and primary liver cancer (PLC) [4]. Aflatoxins are mycotoxins, which are considered to be toxic metabolites, produced mainly in foods by some fungi such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nominus* [5-7]. Mycotoxin is found in foods which stored in hot, humid and unhealthy conditions such as cereals especially maize and rice, some animal source foods such as milk and cheese, hard-shelled fruits such as walnuts, hazelnuts and peanuts, dried fruits and spices which are highly toxic mutagenic, carcinogenic and teratogenic compounds [8-10]. It is estimated that mycotoxins infect up to 25% of the nutrients in the world every year [11]. It has been revealed in studies that there is a relationship between mycotoxin-contaminated food intake and particularly hepatic, gastrointestinal, and carcinogenic diseases [12].

Aflatoxins which are from the mycotoxin group are composed of six main groups namely B_1, B_2, G_1, G_2, M_1 and M_2 [8]. Aflatoxin B_1 (AFB_1) is considered one of the most important mycotoxins due to the potential hepatotoxicity and the worldwide effects [13,14]. AFB_1's high toxicity has emerged as a result of an incident occurred in England in 1960. More than 100.000 turkeys and other poultry fed with peanut pulp contaminated with AFB_1 died. The cause of deaths is stated as the 'Turkey × Disease' originating from the AFB1 [15]. It has been reported that the intake of 2-6 mg/kg/day of AF for a month produces hepatitis in places where deaths are reported in India [16]. It has been observed in a study conducted in Turkey that aflatoxin exposure was significantly higher in viral hepatitis patients than those of healthy subjects and may play an important role in the development of hepatocellular carcinoma [17]. For the development of aflatoxigenic molds 24-35°C temperature and relative humidity above 70% are optimum conditions [18].

Along with the changing living conditions, fast and ready food consumption habits of people increased their tendency towards snack products. The main factor that causes obesity, which has become a major public health problem in many countries, particularly in the USA, is the excessive consumption of energy-intensive foods, especially snack foods. The chips constitute a significant part of the high-energy snack products and they have the very important percentage in terms of snack consumption in our country as well as all over the world [19,20]. The surplus consumption per capita caused the chips market to take a big place in Turkey as a result of the interest and demand of the young population to snacks. Turkey is in the forefront of snack consumption in the world, and chips consumption is the most common. Chips are deep fat fried products with high fat and salt content. The consumption of high amounts of fat and salt increases the risk of coronary heart disease, hypertension, diabetes and cancer [19].

Peanut (*Arachis hypogaea*) is among the most popular oilseeds in the world and has a high lipid content, most of which is formed by unsaturated fatty acids. It is a source of protein with high biological value because it contains essential and non-essential amino acids. In addition to having a good mineral content, it also contains E vitamins and polyphenols with antioxidant properties [21-23]. Due to all these features, peanut is associated with the reduced heart diseases and some cancers and improved weight management [24-26]. Recently, peanut and peanut products are frequently placed in daily diet due to the high health benefits, usability and affordability [27].

Although peanuts are a good source of nutrition, it is well known all over the world that peanuts and their products are the most risky foods in terms of mycotoxin, especially aflatoxin contamination [22,28,29]. Aflatoxin contamination of peanut products causes serious problem for human and animal health and even causes fatal diseases [29-31]. It has been determined in a study that aflatoxins were found with more frequent and higher concentrations in blood serum of children with kwashiorkor than the other malnourished group and control group [32]. In a study which examining the association between the growth retardation and the aflatoxin contaminated food intake, it was reported that there was a significant relationship between the stunting and the aflatoxin [33]. This study is designed to determine the effect of peanuts on the aflatoxin levels of chips and peanut-snack products and to determine the risks in terms of public health, which are added to various snacks for the taste, aroma and flavor, consumed mostly by the younger population at a very high rate. The aim of this study is to determine the amounts of aflatoxin B_1, B_2, G_1 and G_2 in some chips and snacks with peanut consumed in Istanbul.

Materials and Methods

The following chemicals; acetonitrile (ACN), methanol (MeOH), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCI), sodium hydroxide (NaOH) and aflatoxin mix 4 solution (B_1+B_2+G_1+G_2) were obtained from Sigma (St. Louis, MO, U.S.A). Immunoaffinity column (AFLAPREP, Product Code: P07) was obtained from R-Biopharm (Glasgow, UK) for purification of aflatoxins. Teflon tube "tubing" (length: 20 m diameter: 0.25 mm) was purchased from Supelco Analytical. UVA lamp (20 W, 60 cm) was supplied from Sylvania. In this study, all other chemicals were used in high purity.

Preparation of Samples

In this study, 10 different kinds of (classic, spicy, peanut) chips and 4 different kinds of peanut-snack bars were sampled which are obtained from different markets in Istanbul. The samples were homogenized by grinding. Then, 50 g of each sample was weighed and placed in a 250 ml plastic beaker. Then, 5 gr of sodium chloride and 100 ml of distilled water were added and thoroughly mixed in a high speed mixer for 1 min. After that, 150 ml of methanol was added to the prepared mixture and mixed again in the high-speed mixer for 2 min. The mixture was filtered through a filter paper and centrifuged at 4000 rpm for 10 minutes and then adjusted to pH 7.4 with 2M NaOH solution. After purification 5 ml of this obtained liquid was taken and 5 ml of buffer solution was added onto this [34].

Immunoaffinity chromatography

The extract obtained in the preparation of the sample was passed through the immunoaffinity column in a volume of 2 ml per minute with the prepared pump system. After the sample loading was completed, the column was rinsed with 20 ml of buffer solution to remove residual impurities. The toxins were eluted with 1 ml of methanol at a flow rate of approximately 5 ml per minute and then filtered through a 0.22 µm cellulose-based filter and injected into HPLC.

HPLC conditions

The content of aflatoxins was determined by HPLC, consisting of Shimadzu LC 20AT pump with a Shimadzu RF-10AXL fluorescence detector (Shimadzu Corporation, Kyoto, Japan) according to procedure described by [35] with some modifications. The Mobile phase consisted of a mixture of water/acetonitrile/methanol (60/15/30 //v/v/v). Excitation and emission wavelengths 365 nm and 460 nm for aflatoxins, respectively. The separation was performed with a Luna (5 µm, 4.6 × 250 mm), C18 analytical column (Phenomenex, USA) and the flow rate was 1.2 mL/ min. The column oven temperature was maintained at 35°C, the analysis time was 30 min and the injection volume was 50 µl.

Chips samples	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	TOTAL (µg/kg)
Peanut-corn chips	14	16	-	3	33
Potato chips	2	1	-	-	3
Peanut-corn chips	-	-	-	-	-
Sweet corn flavored corn chips	-	-	-	-	-
Garlic bread chips	-	-	-	-	-
Spicy potato chips	-	-	-	-	-
Hot spice aroma flavored corn chips	-	-	-	-	-
Hot Spice Aroma Flavored Corn Chips	-	-	-	-	1
Peanut-corn chips	5	6	-	-	11
Peanut-corn chips	24	15	-	-	39

Table 1: Aflatoxin amounts in chips

Derivatization system

As an alternative to the post-column derivatization system of Kobra Cell, the photochemical derivatization system was established in a laboratory environment. The derivatization system was formed by wrapping a 60 cm long UV-A lamp with a length of 20 m and a 0.5 mm diameter teflon tube.

Results and Discussion

The limit value for aflatoxin B_1, which can be found in human food according to the legal limits of aflatoxins in European Union (EU) member countries, is accepted as 2-4 µg/kg [36,37]. The maximum acceptable value is determined as 5 µg/kg for aflatoxin B_1 and 10 µg/kg for Total aflatoxin (B_1 + B_2 + G_1 + G_2) content for peanut, other oilseeds and spices in the Turkish Food Codex Legislation [38,39]. (Figure 1) shows the chromatogram of aflatoxins mix standard (B_1, B_2, G_1, G_2) and (Figure 2) shows the chromatogram of a peanut-corn chips. (Table 1) shows that aflatoxin B_1 and aflatoxin B_2 were detected in 50% of the analyzed chips products with peanut in the range of 5-24 µg/kg. It has been known for many years that aflatoxin B_1 is the most carcinogenic form of aflatoxin that occurs naturally and has toxigenic properties in living organisms [40,41].

According to the analysis results, the amount of 24 µg/kg aflatoxin B_1 is close to about five times to legal tolerance limits applied in Turkey as 5 µg/kg. When the amount of Total aflatoxin (B_1 + B_2 + G_1 + G_2) in

chips products is considered the results of the analysis obtained as 39µg/kg is approximately twice as much as the tolerable level of 20 µg/kg [42] as determined by the FDA which is an international organization as well as it is approximately quadrupled of 10 µg/kg which is the legal tolerance limits applied in Turkey. The aflatoxin analysis results of the peanut-snack bar samples are given in (Table 2). Aflatoxin was detected in 50% of snack bars in the range of 1-2 µg/kg. However, according to the (Table 1,2) the results of the analysis of 14 samples in total indicate that aflatoxin G2 was found in 14% of the samples. G1 aflatoxin was not detected at all. In Table 1, when the amounts of aflatoxin found in the analysis of chips samples compared with the maximum acceptable values for aflatoxin in the Turkish Food Codex; it can be seen that both the aflatoxin B_1 values and the Total aflatoxin values in 30% of the chips products are above the maximum limits. Table 2 also shows that the amounts of aflatoxin detected in analysed snack bars were below the maximum limits. It is clearly seen that there are serious problems with aflatoxin formation and contamination in the drying and storage stages of peanuts used in the production of snack products and these problems will continue to threaten human health unless precautions are taken. On the other hand, it is known that peanut pellets, which are fully immature, damaged for any reason or damaged by seed coat, have high potential for aflatoxin production [43]. Considering that first quality peanuts are primarily assessed in terms of nut consumption, it is thought that snack manufacturers prefer lower quality peanuts for use in the production as they are commercially economical. As a result of the analysis high levels of aflatoxin in snacks are thought to be the result of the use of lower quality peanut in production which has a high aflatoxin content and it is necessary to show the high precision by the manufacturers and to increase the control of raw materials in terms of aflatoxin in such snack products.

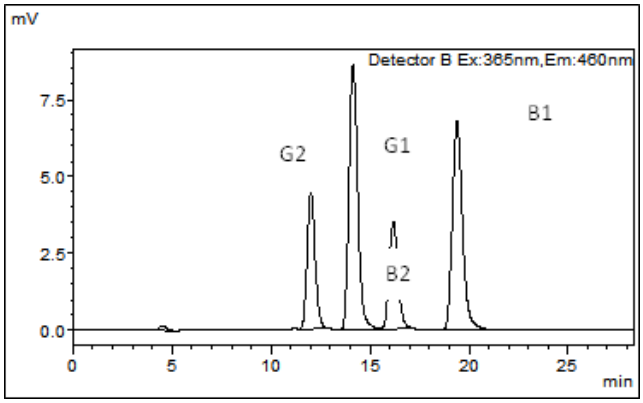

Figure 1: HPLC chromatogram of aflatoxins.

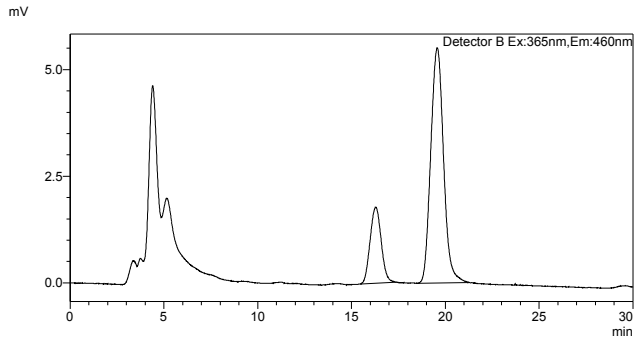

Figure 2: HPLC chromatogram of aflatoxins in peanut-corn chips.

Conclusions

In this study, aflatoxin analysis was performed by HPLC using immunoaffinity chromatography and post-column UV derivatization system in 14 snack samples including 10 different chips and 4 different peanut-snack bars. According to the results of this research, aflatoxin was detected in 50% of samples of snack products containing peanut at different concentrations. However, the amount of aflatoxin detected in the chips samples is much higher than the peanut-snack bar samples and found above the maximum limits. As a result; since aflatoxin is detected in quantities that seriously affect human health in consumed chips, it is suggested to reduce the consumption by considering the other damages of the consumption of chips. It is considered that aflatoxin, which is an important role of the protection and development of the public health, by giving more importance to the studies, should be sampled in adequate amounts and regularly analyzed in chips and peanut-snack products before to be served for consumption and appropriate ones should be presented to consumers. It would be beneficial to improve peanut production techniques, especially drying with modern techniques in the production process as well as to increase the work on the implementation of preventive activities by increasing raw material controls for peanuts used in snack production.

Peanut-snack samples	B1 (µg/kg)	B2 (µg/kg)	G1 (µg/kg)	G2 (µg/kg)	TOTAL (µg/kg)
Rice crispy caramel peanut bar	-	-	-	2	2
Milk chocolate covered peanut caramel nougat bar	-	-	-	-	-
Peanut bar	1	-	-	-	1
Chocolate peanut caramel nougat bar	-	-	-	-	-

Table 2: Aflatoxin amounts in snack bars.

Acknowledgment

This study was carried out in the laboratory of Halal Food R&D center of Istanbul Sabahattin Zaim University.

References

1. World Health Organisation, Cancer, Geneva, Switzerland.
2. Turkish Statistical Institute (2016) Cause of Death Statistics, Ankara, Turkey.
3. Johnson NM, Egner PA, Baxter VK, Sporn MB, Wible RS, et al. (2014) Complete protection against aflatoxin B1-induced liver cancer with a triterpenoid: DNA adduct dosimetry, molecular signature, and genotoxicity threshold. Cancer Prev Res 7: 658-666.
4. Wogan GN (1992) Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res 52: 2114-2118.
5. Steyn P (1998) The biosynthesis of mycotoxins. Rev Med Vet 149: 469-478.
6. Rai M, Jogee PS, Ingle AP (2015) Emerging nanotechnology for detection of mycotoxins in food and feed. Int J Food Scı Nutr 66: 363-370.
7. Erkekoglu P, Kocer-Gumusel B (2014) Aflatoxins, hepatitis and hepatocellular carcinoma: a special focus on turkey's current status. J Liver Dis Transplant 3: 1-8.
8. Kensler TW, Roebuck BD, Wogan GN, Groopman JD (2011) Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. Toxicol Sci 1: 28-48.
9. Chen Y, Kong Q, Chi C, Shan S, Guan B (2015) Biotransformation of aflatoxin B 1 and aflatoxin G 1 in peanut meal by anaerobic solid fermentation of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. Int J Food Microbiol 211: 1-5.
10. de Guadalupe Moctezuma-Zarate M, Carvajal-Moreno M, Espinosa-Aguirre JJ, Gonsebatt-Bonaparte ME, Rojo-Callejas F, et al. (2015) Role of pH in the mutagenicity of aflatoxin B1 in maize tortillas during in vitro human digestion model. J Food Nutr Disor 4: 1-10.
11. Monson MS, Settlage RE, McMahon KW, Mendoza KM, Rawal S, et al. (2014) Response of the hepatic transcriptome to aflatoxin B1 in domestic turkey (Meleagris gallopavo) PLoS One 9: e100930.
12. Fung F, Clark RF (2004) Health effects of mycotoxins: a toxicological overview. J Toxicol Clin Toxicol 42: 217-234.
13. Rawal S, Kim JE, Coulombe Jr R (2010) Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Res Vet Sci 89: 325-331.
14. Ranganathswamy M, Naik ST, Adiver SS (2016) Molecular characterization and management of aspergillus flavus link ex fries in groundnut. Vegetos 29: 121-125.
15. Blount WP (1961) Turkey "X" disease. Turkeys 9: 52-55.
16. Patten RC (1981) Aflatoxins and disease. Am J Trop Med Hyg 30: 422-425.
17. Mizrak D, Engin B, Önder FO, Yener B, Bektaş M, et al. (2009) Aflatoxin exposure in viral hepatitis patients in Turkey. Turk J Gastroenterol 20: 192-197.
18. Arrus K, Blank G, Abramson D, Clear R, Holley RA (2005) Aflatoxin production by aspergillus flavus in Brazil nuts. J Stored Prod Res 41: 513-527.
19. Özçam M, Obuz E, Tosun H (2014) Aflatoxin M1 in tarhana chips. Food Addit Contam Part B Surveill 7: 182-185.
20. Ertop MH, Kutluk K, Çoşkun K, Canlı S (2016) A new approach for production of chips with food ındustry byproducts: gluten enriched chips. Academic Food Journal/Akademik Gıda 14: 398-406.
21. Phan-Thien KY, Wright GC, Lee NA (2010) Genotype-by-environment interaction affects the essential mineral composition of peanut (Arachis hypogaea L.) kernels. J Agric Food Chem 58: 9204-9213.
22. de Camargo AC, Vieira TMFS, Regitano-D'Arce MAB, de Alencar SM, Calori-Domingues MA, et al. (2012) Gamma radiationinduced oxidationand tocopherols decrease in in-shell, peeled and blanched peanuts. Int J Mol Sci 13: 2827-2845.
23. Latif S, Pfannstiel J, Makkar HPS, Becker K (2013) Amino acidcomposition, antinutrients and allergensin the peanut protein fraction obtained by an aqueous enzymatic process. Food Chem 136: 213-217.
24. Jones JB, Provost M, Keaver L, Breen C, Ludy MJ, et al. (2014) A randomized trialon the effects of flavorings on the health benefits of daily peanut consumption. Am J Clin Nutr 99: 490-496.
25. Gonzalez CA, Salas-Salvadó J (2006) The potential of nuts in the preventionof cancer. Br J Nutr 2: 87-94.
26. Moreno JP, Johnston CA, El-Mubasher AA, Papaioannou MA, Tyler C, et al. (2013) Peanut consumptionin adolescentsis associated with improved weight status. Nutr Res 33: 552-556.
27. Cook RW (2014) Peanuts: production, nutritional content and health implications. Nova Science Publishers.
28. Gachomo EW, Mutitu EW, Kotchoni OS (2004) Diversity of fungal species associated with peanuts in storage and the levels of aflatoxins in infected samples. Int J Agric Biol 6: 955-959.
29. Youssef MS, Maghraby-El OMO, Ibrahım YM (2008) Mycobiota and mycotoxins of Egyptian peanut (Arachis hypogeae L.) seeds. Int J Bot 4: 349-360.
30. Bankole SA, Adebanjo A (2003) Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. Afr J Biotechnol 2: 254-263.
31. Craufurd PQ, Prasard PV, Waliyar F, Taheri A (2006) Drought, pod yield, pre-harvest aspergillus infection and aflatoxin contamination on peanut in Niger. Field Crops Res 98: 20-29.
32. Hendrickse RG, Coulter JB, Lamplugh SM, Macfarlane SB, Williams TE, et al. (1982) Aflatoxins and kwashiorkor: a study in Sudanese children. Br Med J 285: 843-846.
33. Gong Y, Hounsa A, Egal S, Turner PC, Sutcliffe AE, et al. (2004) Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ Health Perspect 11: 1334-1338.
34. Aflaprep IFU P07 (2003) R-Biopharm Rhone Ltd, Glasgow, UK.
35. Papadopoulou-Bouraoui A, Stroka J, Anklam E (2002) J AOAC Int 85: 411-416.
36. European Commission-EC (2006) No 118/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. 5-24.
37. European Commission-EC (2010) Commission regulation (EU) no 165/2010 of 26 February 2010, amending regulation (EC) no 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin.
38. (2011) Turkish Food Codex Regulation on Contaminants, Maximum levels for contaminants in foodstuffs.
39. (2012) Turkish Food Codex Regulation on Contaminants.

40. Scheidegger KA, Payne GA (2003) Unlocking the secrets of aspergillus flavus from pathogenicity to functional genomics, J Toxicol Toxin Rev 22: 423-459.
41. Alinezhad S, Tolouee M, Kamalzadeh A, Motalebi AA, Nazeri M, et al. (2011) Mycobiota and aflatoxin B1 contamination of rainbow trout (Oncorhinchus Mykiss) feed with emphasis to aspergillus section flavi. Iran J Fish Sci 10: 363-374.
42. Food and Drug Administration (FDA) (2017) Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. Aflatoxin in Peanuts and Peanut Products Sec
43. Lavkor I, Var I (2017) The control of aflatoxin contamination at harvest, drying, pre storage and storage periods in peanut: the new approach. In aflatoxin-control, analysis, detection and health risks. InTech.