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Detection of Aflatoxins and Proximate Analysis of Selected Different Processed Foods: Biscuits, Cakes and Noodles from Lahore, Pakistan

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Abstract. The current study was conducted to assure quality of biscuits, cakes and noodles by determining aflatoxin B1 and proximate composition of the selected food products. A total of 60 samples (20 each of biscuits, cakes and noodles) were collected from diverse areas of Lahore. Aflatoxins were determined using thin layer chromatography by adopting standard method of the association of official analytical chemists (AOAC). The proximate composition was also determined for selective products. Different parameters like moisture, ash content, crude fiber, crude protein and crude fat in percentages were determined in selected food products. The correlation of aflatoxins was calculated with each factor at significant level ($P<0.05$). Total 19 samples (32.0%) showed presence of aflatoxins. Aflatoxins contamination was observed in 10%, 8.3% and 13.3% contamination in biscuit, cakes and noodles respectively and 89.4% of the total contaminated samples were within permissible limits, while 10.6% (2 samples) were contaminated beyond permissible limits. In cakes, highest moisture content (9.54-19.47%) was observed. Moisture content was 2.23-6.71% in biscuits and 1.21-3.79% in noodles. The minimum and maximum value of ash, crude fiber, crude fat and crude protein were 1.09-2.37%, 0-1.21%, 0.53-9.92% and 2.01-3.91% correspondingly in noodles. The range of ash contents, fiber contents, fat contents, protein contents in cakes 1.12-1.68%, 0-0.97%, 19.76-27.21%, 4.10-5.81%, respectively. In biscuits ash content, crude fibre, fat contents and protein contents range is 0.87-2.98%, 0-1.31%, 19.12-27.45%, 7.02-9.49%, respectively. Positive correlation ($r=0.459$) was found between aflatoxin contamination and moisture contents in cakes. It is needed to monitor aflatoxins levels in processed food due to increase in its occurrence day by day.

Keywords: aflatoxin contamination, proximate composition, thin layer chromatography

Introduction

The diversity and demand of food items is increasing constantly in the food markets. Bakery items are consumed frequently by individuals of all age groups and communal classes (Passos *et al*., 2013). The use and demand of bakery items like biscuits, cakes and noodles has recurrently become popular worldwide (Ahmed and Ashraf, 2019). Normally, biscuits are crispy and hard food products (Agu and Okoli, 2014) whereas cakes are soft products with different ingredients having butter, flour, eggs, baking powder, sugar and other constituents like chocolates, dried and fresh fruits used for flavouring purpose (Kiin-Kabari and Banigo, 2015). These ingredients are used by consumers frequently for making dough (Souki *et al*., 2016). Another cereal based food product such as noodles and pasta have gained recognition because of some properties like taste, ease of cooking, longer shelf life, mechanizations, transportation, economical price and safety (Alemayehu *et al*., 2016; Gulia *et al*., 2014).

In Pakistan such processed foods have shown rapid growth and is accepted by consumers of all ages and socioeconomic groups. Biscuits, cakes and noodles are carbohydrates rich sources and useful for gaining energy (Thorat *et al*., 2018). Different forms of pasta like vermicelli and noodles which are prepared from various starches are also have wheat, alkaline salts and sodium potassium carbonates mixtures alongwith water (Karim and Sultan, 2015; Huang and Lai, 2010).

There are so many factors which make food products vulnerable for use. Some of these are unhygienic conditions, improper temperature and storage conditions (Medina *et al*., 2014). Some other factors like poor products handling, transportation, bad condition of processing machines and contamination of raw materials with different toxins which cannot be easily removed.

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Due to all these factors food products get contaminated and may cause different diseases and deteriorate consumer's health (Suman, 2021; Bullerman and Bianchini, 2007). Different raw materials used in processing of these food products may get contaminated with different fungus metabolites which may cause worse health hazards (Das *et al*., 2021; Boyacioglu, 2019).

Aflatoxins are fungal metabolites which are produced by some species of fungi like *Aspergillus parasiticus, Aspergillus nomius* and *Aspergilus flavus* (Smith and Groopman, 2020). These are most generally linked with different cereals like corn, rice, wheat, groundnuts, dried fruits, cocoa beans, vegetable oils and a variety of various agricultural food products (Khodaei *et al*., 2020; Sarma *et al*., 2017). These cereals provide the base material for dough of biscuits, noodles and cakes (Hussein *et al*., 2019). Aflatoxins cannot be eliminated entirely from the food products during cooking process. If aflatoxins contaminate raw food then these contaminants moves to the finished products and depreciate food products quality (Rodríguez-Carrasco *et al*., 2015; Sakuma *et al*., 2013).

Aflatoxins were identified for the first time in 1960 after the Turkey X disease in England (Tian and Chun, 2017). B1, B2, G1 and G2 are the major aflatoxins types which may contaminate food (Dors *et al*., 2011). The order of toxicity levels of aflatoxins is AFTB1 > AFTG1 > AFTB2 > AFTG2 (Kumar *et al*., 2017). Aflatoxin B1 is most prevalent aflatoxin which according to International Agency for Research on Cancer (IARC) is listed as group 1 carcinogen (Azizi and Rouhi, 2013). Aflatoxins may cause severe, acute and chronic diseases in humans and animals when found beyond permissible levels (Marin *et al*., 2013).

Different countries have different permissible levels for aflatoxins (Cucci *et al*., 2007). In more than 75 countries the permitted levels of aflatoxins are 10 µg/Kg in processed foods (Herzallah, 2009). However, according to EU (European Union) 2 and 4 µg/Kg is allowed level of aflatoxins (EU Regulation, 2006). It is very needed to monitor aflatoxin contamination levels in different food products in order to protect human health from aflatoxin contamination and its severe consequences. The research work is novel as aflatoxins were determined in three highly consumable food products collected from various areas of Lahore Pakistan. The proximate analysis was done to evaluate the composition of various nutrients which may affect aflatoxin concentration in food products. Aflatoxins contamination is directly proportional to moisture contents of food products. Thus, this study was planned to check the presence of aflatoxins contamination alongwith proximate analysis in different biscuits, cakes and noodles marketed in Pakistan.

Material and Methods

Total 60 samples of biscuits, cakes and noodles were collected from general store and local markets of Lahore, Pakistan. Twenty samples of each category were analyzed by using thin layer chromatographic technique as per AOAC method 972.26:2016 for the determination of aflatoxins (AOAC, 2016).

Sample extraction for TLC technique. Samples were crushed and were grinded to powder form for aflatoxin determination 50 g sample was taken in the 500 mL conical flask, 25 mL water, 250 mL chloroform and some quantity of ceilite were added to the flask. The mixture was then shaken by using wrist action shaker for 30 min. The sample was then filtered by using Whatman filter paper, 50 mL of filtrate was taken in 100 mL beaker and was dried on the water bath. After drying 0.5 mL chloroform was added to make dilution of extract. Spotting of different volumes of sample extract was done alongwith standard on thin layer chromatographic plate. The TLC plate was placed in mobile phase containing anhydrous ether and then in second mobile phase containing acetone and chloroform (1:9) by volume. After plate development it was observed under UV cabinet at 365 nm for inspecting the presence or absence of different aflatoxins (Zahra, 2019). The spray of aqueous sulphuric acid (50/50 v/v) was done on the TLC plate for the confirmation of aflatoxins presence.

The aflatoxins were determined by using formula (Nazir *et al*., 2020)

$$\text{Total aflatoxins (µg/Kg)} = S \times Y \times V / W \times Z$$

where:

S = volume of aflatoxins standard in µL of equivalent intensity to Z (µL of sample); Y = aflatoxins standard concentration in µg/mL; V = volume of solvents in µL required to dilute final extract; Z = volume of sample extract in µL require to give fluorescence intensity comparable to that of S = µL of aflatoxins standard; W

= weight of original sample in g contained in final extract; total AFs = concentration of AFB1 + AFB2 + AFG1+ AFG2

Proximate analysis of samples. Proximate analysis including moisture, ash contents, crude fat contents, crude fibre contents and protein contents was determined by using AOAC method 930.05 (AOAC, 2005).

Statistical analysis. The obtained results were statistically analyzed by applying SPSS Minitab software version 17. Analysis of variance (ANOVA) and Pearson correlation was applied at $P<0.05$ to determine the differences in concentrations or to find the correlation between aflatoxin contamination and proximate contents.

Results and Discussion

Presence of aflatoxins. The growth of fungal species producing aflatoxins is maximum in the areas where there is warm temperature and high humid conditions are present (Bbosa *et al*., 2013). Different factors lime moisture (between 18 and 20%), water activity (>0.82), pH (3.0-8.5) and temperature (54-104°F) alongwith different nutrients like carbohydrates, phosphates, zinc, nitrogen sources and further trace metals may affect the aflatoxins concentration in food commodities (Atungulu and Muhammadi-shad, 2019). This is why proximate analysis of food samples was conducted along with aflatoxin analysis.

Out of 60 samples 19 (32.0%) samples showed positive results for aflatoxin contamination including 10% biscuits, 9% cakes and 13% noodles while 68% samples showed negative results for aflatoxins as shown in Fig. 1. Individually 30% biscuits, 25% cakes and 40% noodles samples were contaminated with aflatoxins.

Total aflatoxin incidence ranges in biscuits, cakes and noodles were (1.4-1.69 ppb), (1.43-2.14) and (1.52-3.1) as shown in Table 1. All contaminated samples had aflatoxins within permissible limit of EU regulation except two samples which were contaminated with aflatoxin B1 beyond the limits of EU regulations set for cereal based processed food which is 2 µg/Kg (EU regulation, 2010). There was statistically significant difference at $P<0.05$ was present between the mean of aflatoxin level in three different categories of processed cereal food.

Wheat flour is the main ingredient of the biscuit and cakes which is being mixed with other ingredients like sugar, rice flour, cocoa powder, flavours and oils (Ravi *et al*., 2016). In a report conducted in Pakistan by

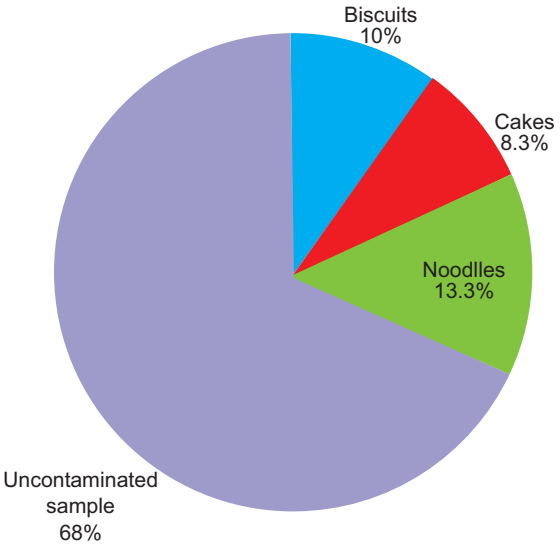

Fig. 1. Aflatoxin contamination percentage.

Table 1. Aflatoxin contamination in different processed foods

Sample	Total no. of samples (N)	Positive samples (n)	Percentage of contaminated sample (%)	Contaminated samples beyond permissible EU limit n (%)	Total aflatoxins in (ppb) (Mean±SD)
~~Biscuits~~	~~20~~	~~6~~	~~30~~	~~0~~	~~1.39±0.04~~ 1.69±0.09
Cakes	20	5	25	1(5)	1.43±0.03 2.14±0.14
Noodles	20	8	40	1(5)	1.52±0.06 3.1±0.21
Total	60	19	32		

Table 2. Proximate analysis of biscuits, cakes and noodles

Sample type	Moisture content	Ash content	Crude fibre	Crude fat	Crude protein
			(%)		
Biscuits	(2.23-6.71)	(0.87-2.98)	(0-1.31)	(19.12-27.45)	(7.02-9.49)
Cakes	(9.54-19.47)	(1.12-1.68)	(0-0.97)	(19.76-27.21)	(4.1-5.81)
Noodles	(1.21-4.72)	(1.09-2.37)	(0-1.21)	(0.53-9.92)	(2.01-3.91)

(Lutfullah and Hussein, 2012), the aflatoxin levels in corn, rice, wheat, sorghum and barley were determined and found the highest value *i.e*. 15.50 µg/Kg aflatoxins in wheat and 13.0 µg/Kg aflatoxins in corn. AFB1 and total AFs were determined in semolina with concentrations of 3.60 µg/Kg-4.55 µg/Kg (Iqbal *et al*., 2014). In a study (Naz *et al*., 2017) aflatoxin contamination of 83% in branded and 91% in non-branded was noted in chocolate samples used in making cakes. Aflatoxins contamination was also found in selective processed food items in Pakistan (Mushtaq *et al*., 2012). 30% samples were found contaminated with aflatoxins. The presence of aflatoxins was also checked in diverse array of pasta such as bucatini, noodles, spaghetti, lasagna and macaroni (Iqbal *et al*., 2014). The presence of aflatoxin was also reported by Mushtaq *et al*. (2012) in 5 instant noodles samples collected from various locations in Pakistan.

Similarly, in republic of Serbia, 34 biscuits were analyzed for aflatoxins presence (Skrbic *et al*., 2017), 8 samples showed positive results for aflatoxins contamination. In Babol city of northern Iran reach by Azizi and Rouhi (2013) conducted a research in which 30 biscuits samples were analyzed, 86.7% samples were found contaminated with aflatoxins, 13.3% biscuits were contaminated with aflatoxins beyond permissible levels. In the present study all the samples were safe for human consumption except 2 (Table 1).

Analysis of proximate content. The percentage of moisture, ash, fat, crude fibre and crude protein was calculated in biscuits, cakes and noodles obtained from different markets in Lahore. The moisture contents were calculated in all 60 samples while ash, fibre, fat and protein were detected in 30 choosen samples which include 10 biscuits, 10 cakes and 10 noodles samples. The range of percentage moisture was 2.23-6.71% for biscuits, 9.54-19.47% for cakes and 1.21-4.72% for noodles. Minimum and maximum value of ash contents in percentage was 0.87-2.98%. Maximum and minimum fibre contents in percentage were 0 -1.31%. Fat contents minimum and maximum percentage value was 19.12 -27.45%. Similarly for protein content minimum and maximum percentage was 7.02-9.49%. The ranges of various proximate contents in cakes were 1.12-1.68% ash, 0-0.97% fibre, 19.76-27.21% fat and 4.1-5.81% protein contents. However, in noodle the range of different parameters calculated were ash (1.09-2.37%), crude fibre (0-1.21%), crude fat (0.53-9.92%) and crude protein (2.01-3.91%) as shown in Table 2.

It was found that moisture contents in the cakes were higher than those in biscuits and noodles. The shelf life of biscuits and noodles is more than cakes due to low moisture as it would not spoil abruptly. The maximum moisture contents are the reason for the aflatoxins contamination in different food samples as depicted by Hassane *et al*. (2017). The fungal growth steadily increases as increase in moisture and temperature of 25% °C.

Correlation between aflatoxins and proximate Analysis. A positive weak correlation was found in current study between contamination of aflatoxins and moisture contents in biscuits and noodles with the linear correlation value *i.e*. $r_{=}$ 0.171 in biscuits and $r_{=}$ 0.168 in noodles as revealed in Table 3. The reason behind may be due to low moisture contents in biscuits as if moisture level increases aflatoxins contamination also increases. The contamination of aflatoxins may be due to the poor handling during manufacturing processing steps (Copetti *et al*., 2012). In a study conducted by (Pesavento *et al*., 2016) in Italy, a negative correlation

Table 3. Pearson correlation between aflatoxin and proximate content

Sample type	Moisture content	Ash content	Crude fibre	Crude fat	Crude protein
Biscuits	0.171	0.110	0.061	0.355	-0.108
Cakes	0.459*	0.037	0.090	0.148	-0.232
Noodles	0.168	0.200	0.196	-0.263	-0.292

* = significant correlation at $P<0.05$.

was found between aflatoxin contamination and moisture levels in chilli powder where, r= -0.17 and m= -779.63. High humidity and high temperature are most favourable conditions for fungal growth. In cakes, a positive correlation, r= 0.459 was observed as moisture contents in cakes was high as shown in the Fig. 1.

In a research in Pakistan conducted by (Zahra, 2019), it was observed that the contamination levels of aflatoxins were high in those wheat flour samples in which moisture contents were high. In current study high moisture contents of cakes reveal significant ($P<0.05$) relation with aflatoxins levels. However, no relation was shown between aflatoxin contamination and moisture for biscuits and noodles. There are other factors like presence of stachyose and arginine may interfere with aflatoxins presence (Liu *et al*., 2016) which considerably enhances the growth of *Aspergillus flavus* in the cereal grains. Aflatoxins and fat contents showed non-significant correlation, r= 0.355. (Ali *et al*., 2009) observed that fat contents and moisture contents if found high in almond seeds.

In present study, a non-significant negative correlation at $P>0.05$ was found between protein in all the samples with the highest r= -0.292 in noodles and r= -0.232 in cakes samples. These results were equivalent with the results of (Alamu *et al*., 2018) which showed significant ($P<0.05$) negative correlation for protein in Chipata samples where (r= -.01877 and Monze where r= -.40105). Therefore, it is concluded from the studies that those food samples which contain high levels of aflatoxin contamination had low protein levels. There is non-significant positive correlation between ash contents of biscuits, cakes and noodles *i.e*. r= 0.110, r= 0.037 and r= 0.200 as shown in Table 3.

Conclusion

Aflatoxins were detected in biscuits, cakes and noodles collected from different areas of Lahore, Pakistan. Overall, 32.0% samples were found to be contaminated with aflatoxins. Highest level of aflatoxins was found in noodles than in biscuits and cakes. Fifty eight samples showed presence of toxin within permissible limit prescribed by EU. The positive correlation was found between aflatoxins contamination and moisture contents in cakes, while in case of biscuits and noodles, no such correlation was found. Although, the percentage contamination level of aflatoxins is low but it is needed to monitor aflatoxins presence in processed foods like biscuits, cakes and noodles. It is recommended to adopt quality assurance, quality control and continual improvement from raw material to processed food in order to avoid aflatoxins contamination to ensure food safety and security.

Conflict of Interest. The authors declare that they have no conflict of interest.

References

Agu, H.O., Okoli, N.A. 2014. Physico-chemical, sensory and microbiological assessments of wheat-based biscuit improved with beniseed and unripe plantain. *Food Science and Nutrition*, **2:** 464-469.

Ahmed, H.A.M., Ashraf, S.A. 2019. Physico-chemical, textural and sensory characteristics of wheat flour biscuits supplemented with different levels of whey protein concentrate. *Current Research in Nutrition and Food Science Journal*, **7:** 761-771.

Alamu, E.O., Gondwe, T., Akello, J., Sakala, N., Munthali, G., Mukanga, M., Maziya-Dixon, B. 2018. Nutrient and aflatoxin contents of traditional complementary foods consumed by children of 6-24 months. *Food Science and Nutrition*, **6:** 834-842.

Alemayehu, D., Desse, G., Abegaz, K., Desalegn, B.B., Getahun, D. 2016. Proximate, mineral composition and sensory acceptability of homemade noodles from stinging nettle (*Urtica simensis*) leaves and wheat flour blends. *International Journal of Food Science and Nutrition Engineering*, **6:** 55-61.

Ali, E., Saleemullah, F.A., Khan, B.A., Zubair, A. 2009. Aflatoxin contamination and mineral profile of almond seeds. *Mycopath*, **7:** 39-44.

AOAC, 2016. *Official Methods of Analysis, 20th edition*, AOAC 972.26:2016, Association of Official Analytical Chemists, Washington DC, USA.

AOAC, 2005. *Official Methods of Analysis of the Association of Analytical Chemist International*, 18th edition, Gaithersburg, MD: Association of Official Chemists Methods.

Atungulu, G., Mohammadi-Shad, Z. 2019. Reference on mycotoxins occurrence, prevalence and risk assessment in food systems. In: *Novel Technologies and Systems for Food Preservation*, pp. 294-343, IGI Global, USA.

Azizi, I.G., Rouhi, S. 2013. The comparison of total fumonisin and total aflatoxin levels in biscuit and cookie samples in Babol city, northern Iran. *Iranian*

Journal of Public Health, **42:** 422.

Bbosa, G.S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W.W., Kyegombe, D.B. 2013. Review of the biological and health effects of aflatoxins on body organs and body systems. *Aflatoxins Recent Advances and Future Prospects*, **12:** 239-265.

Boyacioglu, H. 2019. *The Pathogen Risks Lurking in Baked Foods*. Food business News. https://www.foodbusinessnews.net/articles/14105-the-pathogen-risks-lurking-in-baked-foods

Bullerman, L.B., Bianchini, A. 2007. Stability of mycotoxins during food processing. *International Journal of Food Microbiology*, **119:** 140-146.

Copetti, M.V., Iamanaka, B.T., Pereira, J.L., Lemes, D.P., Nakano, F., Taniwaki, M.H. 2012. Co-occurrence of ochratoxin A and aflatoxins in chocolate marketed in Brazil. *Food Control*, **26:** 36-41.

Cucci, C., Mignani, A.G., Dall'Asta, C., Pela, R., Dossena, A. 2007. A portable fluorometer for the rapid screening of M1 aflatoxin. *Sensors and Actuators B: Chemical*, **126:** 467-472.

Das, S., Singh, V.K., Dwivedy, A.K., Chaudhari, A.K., Dubey, N.K. 2021. Nano-structured *Pimpinella anisum* essential oil as novel green food preservative against fungal infestation, aflatoxin B1 contamination and deterioration of nutritional qualities. *Food Chemistry*, **344:** 128574.

Dors, G.C., Caldas, S.S., Feddern, V., Bemvenuti, R.H., Cristina, H., Hackbart, S., deSouza, M.M., Oliveira, M.S., Garda-Buffon, J., Primel, E.G., Badiale-Furlang, E. 2011. *Aflatoxins: Contamination, Analysis and Control*, 15th edition, pp. 1-26, Institute Federal Catarinese (IFC), Brazil.

Gulia, N., Dhaka, V., Khatkar, B.S. 2014. Instant noodles: processing, quality and nutritional aspects. *Critical Reviews in Food Science and Nutrition*, **54:** 1386-1399.

Hassane, A.M.A., El-Shanawany, A.A., Abo-Dahab, N.F., Abdel-Hadi, A.M., Abdul-Raouf, U.M., Mwanza, M. 2017. Influence of different moisture contents and temperature on growth and production of aflatoxin B1 by a toxigenic *Aspergillus flavus* isolate in wheat flour. *Journal of Ecology of Health and Environment*, **5:** 77-83.

Herzallah, S.M. 2009. Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Journal of Food Chemistry*, **114:** 1141-1146.

Huang, Y.C., Lai, H.M. 2010. Noodle quality affected by different cereal starches. *Journal of Food Engineering*, **97:** 135-143.

Hussien, A.M.S., Badr, A.N., Naeem, M.A. 2019. Innovative nutritious biscuits limit aflatoxin contamination. *Pakistan Journal of Biological Sciences*, **22:** 133-142.

Iqbal, S.Z., Rabbani, T., Asi, M.R., Jinap, S. 2014. Assessment of aflatoxins, ochratoxin A and zearalenone in breakfast cereals. *Food Chemistry*, **157:** 257-262.

Iqbal, S.Z., Asi, M.R., Jinap, S., Rashid, U. 2014. Detection of aflatoxins and zearalenone contamination in wheat derived products. *Food Control*, **35:** 223-226.

Karim, R., Sultan, M.T. 2015. *Yellow Alkaline Noodles: Processing Technology and Quality Improvement*, pp. 5-6, Springer briefs in food, health and nutrition, Heidelberg, New York, USA.

Khodaei, D., Javanmardi, F., Khaneghah, A.M. 2021. The global overview of the occurrence of mycotoxins in cereals: a three-year survey. *Current Opinion in Food Science*, **39:** 36-42.

Kiin-Kabari, D.B., Banigo, E.B. 2015. Quality characteristics of cakes prepared from wheat and unripe plantain flour blends enriched with bambara groundnut protein concentrate. *European Journal of Food Science and Technology*, **3:** 1-10.

Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K., Kang, S.G. 2017. Aflatoxins: a global concern for food safety, human health and their management. *Frontiers in Microbiology*, **7:** 2170.

Liu, J., Sun, L., Zhang, N., Zhang, J., Guo, J., Li, C., Rajput, S.A., Qi, D. 2016. Effects of nutrients in substrates of different grains on aflatoxin B1 production by *Aspergillus flavus*. *BioMed Research International*, **2016:** 7232858.

Lutfullah, G., Hussain, A. 2012. Studies on contamination level of aflatoxins in some cereals and beans of Pakistan. *Food Control*, **23:** 32-36.

Marin, S., Ramos, A.J., Cano-Sancho, G., Sanchis, V. 2013. Mycotoxins: occurrence, toxicology and exposure assessment. *Food and Chemical Toxicology*, **60:** 218-237.

Medina, A., Rodríguez, A., Sultan, Y., Magan, N. 2015. Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production. *World Mycotoxin Journal*, **8:** 171-179.

Mushtaq, M., Sultana, B., Anwar, F., Khan, M.Z., Ashrafuzzaman, M. 2012. Occurrence of aflatoxins in selected processed foods from Pakistan.

International Journal of Molecular Sciences, **13:** 8324-8337.

Naz, N., Kashif, A., Kanwal, K., Ajaz, H. 2017. Incidence of mycotoxins in local and branded samples of chocolates marketed in Pakistan. *Journal of Food Quality*, **2017:** 1947871.

Nazir, A., Kalim, I., Imran, M., Bilal, M.A., Zahra, N., Ahmad, A., Iqbal, M., Fazal, U., Ehtisham-ul-Haque, S. 2021. Incidences and bio-detoxification of aflatoxins in rice and cattle feed crops under different agro-ecological zones. *Polish Journal of Environmental Studies*, **30:** 1-7.

Passos, M.E.A.D., Moreira, C.F.F., Pacheco, M.T.B., Takase, I., Lopes, M.L.M., Valente-Mesquita, V.L. 2013. Proximate and mineral composition of industrialized biscuits. *Food Science and Technology*, **33:** 323-331.

Pesavento, G., Ostuni, M., Carmela,C., Rossi, S., Capei, R., Antonella, L.N. 2016. Mycotic and aflatoxin contamination in *Myristica fragrans* seeds (nutmeg) and *Capsicum annum* (Chilli), packaged in Italy and commercialized worldwide. *Journal of Preventive Medicine and Hygiene*, **57:** 102-109.

Ravi, K., Samsher, C., Suresh, S. 2016. Studies on proximate analysis of biscuits using multigrain flours during ambient condition. *International Journal of Food Science and Nutrition*, **1:** 39-41.

Rodríguez-Carrasco, Y., Fattore, M., Albrizio, S., Berrada, H., Mañes, J. 2015. Occurrence of *Fusarium mycotoxins* and their dietary intake through beer consumption by the European population. *Food Chemistry*, **178:** 149-155.

Sakuma, H., Watanabe, Y., Furusawa, H., Yoshinari, T., Akashi, H., Kawakami, H., Saito, S., Sugita-Konishi, Y. 2013. Estimated dietary exposure to mycotoxins after taking into account the cooking of staple foods in Japan. *Toxins*, **5:** 1032-1042.

Sarma, U.P., Bhetaria, P.J., Devi, P., Varma, A. 2017. Aflatoxins: implications on health. *Indian Journal of Clinical Biochemistry*, **32:** 124-133.

Škrbiæ, B., Antiæ, I., Cvejanov, J. 2017. Determination of mycotoxins in biscuits, dried fruits and fruit jams: an assessment of human exposure. *Food Additives and Contaminants: Part A*, **34:** 1012-1025.

Smith, J.W., Groopman, J.D. 2020. Translating DNA adduct knowledge to human prevention trials. In: *Proceeding of DNA Damage, DNA Repair and Disease*, pp. 361-381, Royal Society of Chemistry, New York, USA.

Souki, G.Q., Reis, V.C., Moura, L.R.C. 2016. The behaviour of bakery consumers. *Organizações Rurais and Agroindustriais*, **18:** 1-12.

Suman, M. 2021. Last decade studies on mycotoxins' fate during food processing: an overview. *Current Opinion in Food Science*, **41:** 70-80.

Thorat, A.V., Kardile, N.B., Wagh, R.P. 2018. Physiochemical and sensory analysis of noodles fortified with cowpea and pomegranate peel powder. *International Journal of Chemical Studies*, **6:** 3051-3054.

Tian, F., Chun, H.S. 2017. Natural products for preventing and controlling aflatoxin contamination of food. *Aflatoxin: Control, Analysis, Detection and Health Risks*, pp. 13-44, Open access Peer received DOI:105772/intechopen.68-413

Zahra, N. 2019. Effect of moisture content on aflatoxin B1 production in wheat flour samples collected from Lahore, Pakistan. *Pakistan Journal of Analytical and Environmental Chemistry*, **20:** 184-189.