International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 12-08-2023, Accepted: 27-08-2023, Published: 13-09-2023
Volume 5, Issue 3, 2023, Page No. 61-66

A review Mycotoxins: Extraction, characterization and analysis

Sowmya K L, Ramalingappa B
Research Scholar, Department of Microbiology, Davangere University, Shivagangotri, Davangere, Karnataka, India
Professor, Department of Microbiology, Davangere University, Shivagangotri, Davangere, Karnataka, India

Abstract
Mycotoxins are a diverse group of secondary, occurring naturally, and essentially unavoidable compounds produced by fungi. They contaminate a range of bakery foods, including bread, buns, biscuits, Peda, and cake, at any point throughout the baking process. Mycotoxin-contaminated food and feed can make people and animals poisonous in an acute or chronic way. The threat to public health has necessitated the development of methods for the investigation and mycotoxins analysis in food items. Because mycotoxins have a wide range of structural variations, excellent chemical stability, and low concentrations in test samples, they require powerful, effective, and intelligible detection procedures. This review describes the techniques that have been used successfully to identify and detect different mycotoxins in food products, including chromatographic and immunochemical methods as well as cutting-edge, alternative methods like biosensors, electronic noses, and molecularly imprinted polymers. To emphasize the significance of sampling and sample handling in the analytical process, these procedures have been covered in detail.

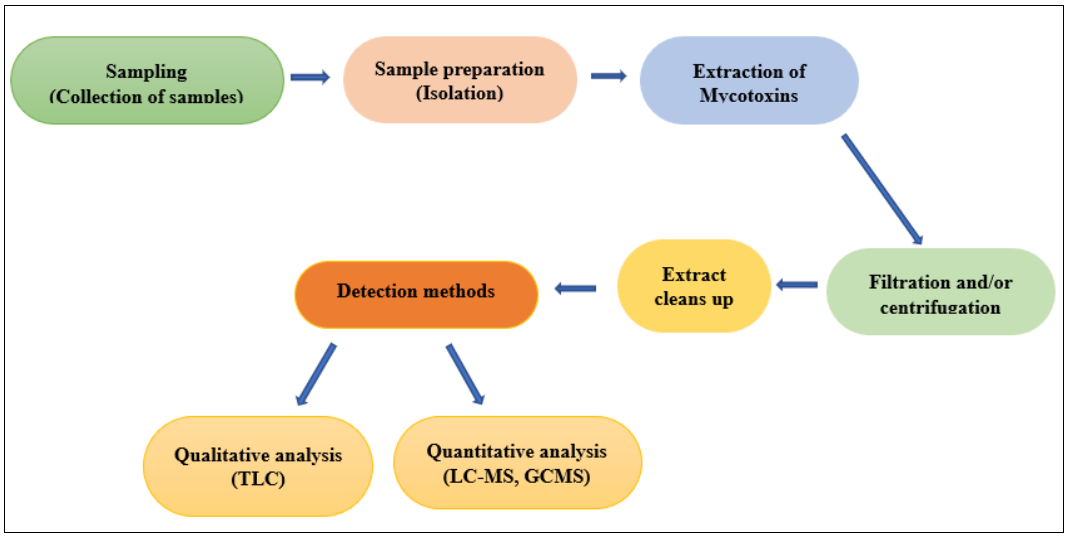
Keywords: Mycotoxins, thin layer chromatography, liquid chromatography, gas chromatography, ELISA

Introduction

Mycotoxins are secondary metabolites of filamentous fungus with a low molecular mass (MW 700 Da) that are toxic to both humans and animals [1]. A large number of distinct fungus species are responsible for generating more than 400 distinct mycotoxins with diverse chemical structures and properties [2]. *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* are the principal genera of mycotoxigenic fungus [3]. Aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), patulin (PAT), fumonisins (FUMs), and trichothecenes (TCs), including deoxynivalenol (DON) and T-2 toxin (T-2), are some of the most dangerous mycotoxins. Mycotoxins have been found to be present in a lot of bakery foods like bread, cake, buns, and biscuits. The growth and mycotoxin production processes in various fungi species can be influenced by a variety of factors. Temperature, humidity, water activity (aw), pH, nutrients, substrate type, amount of inoculation, physiological condition, and microbial interactions are a few examples of environmental variables [4]. Toxin production is a possibility throughout all stages of food product manufacturing, packaging, distribution, and storage [5]. The manufacturing of food and feed happens to be where mycotoxin contamination occurs most frequently.

The advent of food processing methods such grinding, baking, boiling, roasting, frying, and pasteurization has led to the chemical and thermal stability of a large number of mycotoxins [6]. A variety of harmful characteristics define mycotoxins. There may be severe acute responses or long-term consequences, depending on the amount or length of exposure. The World Health Organisation (WHO), the European Commission (EC), the Food and Agriculture Organisation of the United Nations (FAO), and Other national and international institutions and organisations have discovered potential health risks for people and animals related to ingesting mycotoxin-contaminated food or feed. To address this problem, regulatory limits for the main mycotoxin classes and a few specific mycotoxin types have been developed [7]. For mycotoxins in food and feed such DON and AFs, the FDA has produced literature lists [8] and guidance materials [9]. Regarding the maximum quantity of mycotoxins in various foods, the EC has created extensive rules [10]. Mycotoxins in food and feed are governed by stringent international laws created by the FAO [11]. Additionally, the toxicity, exposure, and daily limitations of fumonisins and AFs as food pollutants have been thoroughly assessed in the report of the Joint FAO/WHO Expert Committee on Food Additives [12]. Numerous analytical techniques have been developed to identify and measure mycotoxins in food samples as a result of all of these attempts to set mycotoxin limits and standards. Chromatographic methods, immunoassay-based techniques, and quick strip screening tests are some of the approaches that have been shown effective in the measurement of mycotoxins in products from bakeries [13]. Despite the enormous advancements achieved in this area, there are still many difficulties and drawbacks to these analytical techniques that need to be resolved. Special extraction, cleanup, and detection techniques are needed due to the mycotoxins' chemical diversity, co-occurrence, changing concentrations in food commodities, and complex food matrices that include mycotoxin contamination [14]. This study covers the methodology and distinctive, innovative methods used for mycotoxin analysis and detection in a variety of foods. General flow chart for the detection of mycotoxins is shown in Table 1.

Table 1: Methods in mycotoxins detection.



Sampling and Preparation of sample

Prior to mycotoxin identification in food samples, several different techniques are used including sample preparation and sampling. Extraction and cleanup are both included in sample preparation. Both are essential and are inextricably linked. An accurate determination of mycotoxins is made possible by properly carrying out each of these processes [15].

1. Sampling

Sampling is crucial in evaluating mycotoxin levels since mycotoxigenic fungi do not even grow on the substrate and because it is difficult to get a representative bulk sample. In addition, the mycotoxin contaminations that are already present in natural samples identical. In order to standardise the mycotoxin analysis sampling techniques, Commission Regulation [16] established the sampling and analysis methodologies for the official regulation of the mycotoxin levels in foodstuffs. Inadequate sampling is associated with mycotoxin estimate mistakes that typically lead to an underestimation. Inaccurate information may be given to risk assessors and managers if sampling is done for monitoring or surveillance. For inspection purposes, inaccurate sampling might be problematic [17].

2. Grinding and Mixing

To expedite the chemical reaction process of extraction and increase the possibility of identifying the mycotoxins, the sample should be homogenised until it resembles whole wheat flour or powder [18]. It should also be processed to a final particle size. The sample has to be blended when homogeneity has been achieved. The slurry mixing procedure seems to be a good choice in light of the study that has been done and the comparison of the different approaches. Very tiny particle sizes and hence homogenous samples with the lowest variation ratio were produced by this technique [19].

3. Extraction and Purification

The first stage of preparing a sample is extraction, which involves utilising suitable solvents to get rid of mycotoxins from contaminated food and feed samples. Initially established, the Fast, Easy, Cheap, Efficient, Rugged, and Effective approach enables the simultaneous detection of multiple groups of mycotoxins in diverse frameworks [20]. This technique involves a liquid-liquid extraction followed by an extraction with acetonitrile water. This process begins with an extraction using acetonitrile water, which is followed by the addition of inorganic salts to induce liquid-liquid partitioning. Because of this, mycotoxins are transported to the organic phase while certain polar matrix components remain in the aqueous layer. A dispersive solid phase extraction is then used to eliminate additional matrix compounds from the organic phase [21].

The next extraction method, known as liquid-liquid extraction (LLE), is based on the toxin's different solubilities in the aqueous phase and the immiscible organic phase. While the chemical extraction is carried out in one solvent, the residual matrix is maintained separate [22]. LLE has been utilised to concurrently evaluate AFs and OTA [23].

The mycotoxin may be extracted from solid matrices of different consistencies using the straightforward technique of liquid-solid extraction (SLE). Weighing the homogenised sample, the fundamental steps in the extraction process are adding the extraction solvent and mixing it in a shaker [24]. It has been demonstrated that this technique works to remove different mycotoxins from grains. The same process as SLE, accelerated solvent extraction (ASE) or pressurised liquid extraction (PLE), is carried out in a pressure-resistant vessel at higher pressure and temperature [25, 26]. To enhance the extraction of analytes from the matrix, these techniques utilise common solvents at high temperatures (100–180 °C) and pressures (1500–2000 psi) [27].

The Supercritical Fluid Extraction (SFE) technique is the next technique. By using supercritical CO_2, SFE can reduce and eventually do away with the need for organic solvents. The SFE method is often used to extract non-polar chemical components and has been used to identify ZEA in maize flour [28]. All extraction methods, solvents, advantages, and disadvantages are included in Table 2.

Table 2: Extraction techniques, solvent, benefits, and drawbacks.

Method	Solvent	Benefits	Drawbacks	References
Liquid-Liquid extraction	Cyclohexane, Hexane,	Beneficial for preparations on a modest size	may not always give an analyte that is adequately clean, is time-consuming, and could result in sample loss due to adsorption onto the glassware.	[45, 49, 56]
Liquid-Solid Extraction	methanol/water, Acetonitrile/water,	Least quantity of solvent	SLE by itself may not be sufficient to remove certain mycotoxins without interfering, necessitating further purification processes.	[47, 49]
Pressurized Liquid Extraction	acetonitrile/methanol, Acetonitrile/water,	The ability to automate the extraction process, increase extraction efficiency in less time, and use less extraction solvent	expensive instruments	[47, 49, 57]
Supercritical Fluid Extraction	Acetonitrile supercritical CO_2 fluid	Quick, minimal solvent volumes, and temperature-sensitive analyte extraction	Inadequate recoveries, high co-extract concentrations, and high costs	[45, 49]

To be released, the mycotoxins need to be separated from the matrix. To reduce matrix effects and remove everything that can obstruct the next mycotoxin detection, the extract must be carefully cleaned. Purification of the extract can increase its sensitivity and selectivity while also increasing the precision and accuracy of the measurement. The most popular methods for cleaning up mycotoxins are solid phase extraction (SPE) and immunoaffinity columns (IAC), which are rapid, efficient, consistent, and offer a wide range of selectivity [29]. In order to remove contaminants and capture the mycotoxins, the solid absorbents (where the mycotoxins are absorbed) employed in the SPE method are commonly packed in cartridges [30]. SPE is a rapid, efficient, and reproducible method, but it has a number of limitations, including the challenge of detecting all mycotoxins with a single cartridge. Additionally, a variety of elements, such the kind of solvent employed or the sample's pH and ionic strength, may affect effectiveness [31].

Detection and Analysis of Mycotoxins

Numerous methods have been used in order to find mycotoxins in food and feed ever since the first ones were found [32]. The utilisation of several distinct chromatography types, including thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC), in combination with diverse detectors including diode array, fluorescence, and UV, is what primarily accounts for the supremacy of chromatographic methods. Both gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been used to identify mycotoxins with success [33]. When a rapid mycotoxin analysis is required, immunoassay methods such lateral flow immunoassay (LFIA) [34, 35] and enzyme-linked immunosorbent assay (ELISA) [36, 37] are essential. Biosensors also appear to be a useful method for identifying mycotoxins in food [38, 39].

1. Chromatography Techniques

1.1 Thin Layer Chromatography

Thin layer chromatography is an established form of mycotoxin detection since it allows for the cost-effective screening of several samples [40]. Thin layer chromatography consists of a stationary phase consisting of cellulose, alumina or silica, that is immobilised on an inert matrix made of glass or plastic. Methanol, acetonitrile, and water combinations make up the mobile phase, which transports the sample from the solid stationary phase to the liquid phase [41]. This technique is reliable for finding mycotoxins. It is crucial in the investigation of several mycotoxins due to its inexpensiveness and luminous spots under UV light. This method was created for the qualitative [42, 43] and quantitative [44-45] analysis of mycotoxin. However, due to TLC's weak sensitivity and precision, quantification is quite difficult [46]. The preparation of the sample and the type of cleanup technique is another essential element that heavily depends on the characteristics and mycotoxin type.

1.2 Liquid Chromatography

No matter their biological activity or chemical composition, LC enables the simultaneous detection of several mycotoxins. An analytical column and a mobile phase are used to separate analytes from the matrix ingredients. The isolation and detection of highly polar, non-volatile, and thermally labile mycotoxins are also done using this technique [47]. Mycotoxin analysis mostly uses HPLC with a variety of adsorbents, depending on the chemical and physical characteristics of the mycotoxin. Most mycotoxins detection methods are rather similar to one another. The most popular detectors in HPLC are the UV-visible (UV), fluorescent (FLD), and MS (single mass spectrometry and tandem MS (MS/MS)) detectors, which depend on the presence of a chromophore in the molecules. Some poisonous compounds, such AFs and OTA, already glow in their natural state and can be discovered in HPLC-FLD. HPLC-FLD is the most used technique for locating OTA in various matrices. LC-MS/MS offers superior selectivity and sensitivity, more confidence of analytes identification, and a wider range of matrices as compared to conventional methods using conventional detectors [48]. The majority of mycotoxigenic fungi have the capacity to produce several distinct mycotoxins at once.

1.3 Gas Chromatography (GC)

GC depends on the differential partitioning of analytes between the two GC column phases. The many chemical components of the sample are dispersed across stationary and mobile phases. After the separation process, volatile chemicals are located using a mass spectrometer, an electron capture detector (ECD), or a flame ionisation detector (FID). Mycotoxins are seldom analysed by GC because of the analytes' poor volatility and high polarity. As a result, mycotoxins in milled grain-based products have been detected using the GC-MS/MS approach [49].

ELISA

In addition to the sensitive but challenging and expensive chromatographic methods, immunochemical approaches like ELISA offer rapid and simple screening procedures for on-site mycotoxin detection [50, 51]. ELISA allows for the simultaneous testing of several samples and has a precise detection rate [52]. It is a high-throughput test with reduced sample volume requirements and fewer clean-up procedures than chromatographic technologies like HPLC or TLC. Mycotoxins have been frequently detected in a range of foods using the ELISA method.

Innovative Mycotoxin Analysis and Detection Technologies

Several alternative techniques that have been generated and may be helpful in mycotoxin detection have been developed in addition to the traditional techniques mentioned above. However, outside of the study fields, these techniques have not been extensively employed and have limited utility. Additionally, they need additional validation and verification from reputable agencies as the European Standardisation Committee (CEN), International Organisation for Standardisation (ISO), or Association of Official Analytical Chemists (AOAC) [29]. This technique involves following detection methods like the Aggregation-Induced Emission, Fluorescent Polarization, Molecularly Imprinted Polymers, Electronic nose.

Conclusions

Bakery food Contamination with mycotoxins resulted in establishing their acceptable in food. The preparation of the sample using various extraction and purification techniques is a crucial stage in the analysis of mycotoxin. The most used analytical techniques for determining mycotoxins are still TLC and LC. High sensitivity and dependability are ensured by chromatography methods. New possibilities in mycotoxin identification have been made possible by recent developments in detection and analysis technology and the creation of innovative methods as Aggregation-Induced Emission, Fluorescent Polarisation, Molecularly Imprinted Polymers, and Electronic Nose.

Acknowledgement

The work was carried out in the Bioprocess and Fermentation Technology Laboratory, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka. Author is greatful to research supervisor, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka, India for providing well equipped laboratory facility to carry out the present research work.

Funding

The authors declare that there is no funding.

Conflict of interest

The authors declare that there is no Conflict of Interest.

References

1. Liew WPP, Mohd-Redzwan S. Mycotoxin: Its Impact on Gut Health and Microbiota. Front. Cell. Infect. Microbiol,2018:8:60.
2. Palumbo R, Crisci A, Venancio A, Cortinas Abrahantes J, Dorne JL, Battilani P, *et al*. Occurrence and co-occurrence of mycotoxins in cereal-based feed and food. Microorganisms,2020:8:4.
3. Zain ME. Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc,2011:15:129-144.
4. Agriopoulou S, Stamatelopoulou E, Varzakas T. Advances in Occurrence, Importance, and Mycotoxin Control Strategies: Prevention and Detoxification in Foods. Foods,2020:9:137.
5. Karlovsky P, Suman M, Berthiller F, De Meester J, Eisenbrand G, Perrin I, *et al*. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Res,2016:32:179-205.
6. Bullerman L. Bianchini A. Stability of mycotoxins during food processing. Int. J. Food Microbiol,2007:119:140-146.
7. Krska R, Schubert-Ullrich P, Molinelli A, Sulyok M, MacDonald S, Crews C. Mycotoxin analysis: An update. Food Addit. Contam,2008:25:152-163.
8. The US Food and Drug Administration. Guidance for Industry: Action Levels for Poisonous or Deletorious Substances in Human Food and Animal Feed. Available online: https://www.fda.gov/regulatory-information/search-fda-guidancedocuments/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla (accessed on 3 February 2021).
9. The US Food and Drug Administration. Guidance or Industry and FDA: Advisory Levels for Deoxynivalenol (DON) in Finished Wheat Products for Human Consumption and Grains and Grain By-Products Used for Animal Feed. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-and-fda-advisorylevels-deoxynivalenol-don-finished-wheat-products-human (accessed on 3 February 2021). Molecules,2021:26:3981 14 of 19.
10. The European Commission. Commission Regulation (EC) No 1881/2006 of 19 December Setting Maximum Levels or Certain Contaminants in Foodstuff. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1881-20140701&from=EN (accessed on 3 February 2021).
11. Food and Agriculture Organization of the United Nations. Worldwide Regulations for Mycotoxins in Food and Feed in 2003. Available online: http://www.fao.org/3/y5499e/y5499e00.htm (accessed on 3 February 2021).
12. World Health Organization. Evaluation of Certain Contaminants in Food: Eighty-Third Report of the Joint FAO/WHO Expert Committee on Food Additives. Available online: https://apps.who.int/iris/bitstream/handle/10665/254893/9789241210027eng.pdf;jsessionid=4E6EBA0A0F5160EC5DC55868695CF4E1?sequence=1 (accessed on 3 February 2021).
13. Zhang L, Dou XW, Zhang C, Logrieco AF, Yang MH. A Review of Current Methods for Analysis of Mycotoxins in Herbal Medicines. Toxins,2018:10:65.

14. Hajslova J, Zachariasova M, Cajka T. Analysis of Multiple Mycotoxins in Food. Mol. Biol,2011:747:233-258.
15. Tittlemier SA, Cramer B, Dall'Asta C, Iha MH, Lattanzio VMT. Maragos C. Solfrizzo M. Stranska CM. Stroka J. Sumarah M. Developments in mycotoxin analysis: An update for 2018–19. World Mycotoxin J,2020:13:3-24.
16. European C. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off. J. Eur. Union,2006:70:12-34.
17. Miraglia M, De Santis B, Minardi V, Debegnach F, Brera C. The role of sampling in mycotoxin contamination: A holistic view. Food Addit. Contam,2005:22:31-36.
18. Nakhjavan B, Ahmed NS, Khosravifard M. Development of an Improved Method of Sample Extraction and Quantitation of Multi-Mycotoxin in Feed by LC-MS/MS. Toxins,2020:12:462.
19. Spanjer MC, Scholten JM, Kastrup S, Jorissen U, Schatzki TF, Toyofuku N. Sample comminution for mycotoxin analysis: Dry milling or slurry mixing? Food Addit. Contam,2006:23:73-83.
20. Pereira VL, Fernandes JO, Cunha SC. Comparative assessment of three cleanup procedures after QuEChERS extraction for determination of trichothecenes (type A and type B) in processed cereal-based baby foods by GC–MS. Food Chem,2015:182:143-149.
21. Gonzalez-Jartín JM, Alfonso A, Rodríguez I, Sainz MJ, Vieytes MR, Botana LM. A QuEChERS based extraction procedure coupled to UPLC-MS/MS detection for mycotoxins analysis in beer. Food Chem,2019:275:703-710.
22. Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: A review. Anal. Chim. Acta,2009:632:168-180.
23. Andrade PD, Da Silva JLG, Caldas ED. Simultaneous analysis of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquid chromatography/fluorescence after liquid–liquid extraction with low temperature purification (LLE–LTP). J. Chromatogr. A,2013:1304:61-68.
24. Xie L, Chen, M, Ying Y. Development of Methods for Determination of Aflatoxins. Crit. Rev. Food Sci. Nutr,2016:56:2642-2664.
25. Miklos G, Angeli C, Ambrus A, Nagy A, Kardos V, Zentai A, *et al*. Detection of Aflatoxins in Different Matrices and Food-Chain Positions. Front. Microbiol,2020:11:1916.
26. Rico-Yuste A, Gomez-Arribas LN, Perez-Conde MC, Urraca JL, Moreno-Bondi MC. Rapid determination of *Alternaria* mycotoxins in tomato samples by pressurised liquid extraction coupled to liquid chromatography with fluorescence detection. Food Addit. Contam. Part A,2018:35:2175-2182.
27. Razzazi-Fazeli E, Reiter E. Sample preparation and clean up in mycotoxin analysis: Principles, applications and recent developments. Determ. Mycotoxins Mycotoxigenic Fungi. Food Feed, 2011, 37.
28. Zougagh M, Rios A. Supercritical fluid extraction of macrocyclic lactone mycotoxins in maize flour samples for rapid amperometric screening and alternative liquid chromatographic method for confirmation. *J. Chromatogr. A*,2001:177:50-57.
29. Alshannaq A, Yu JH. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *Int. J. Environ. Res. Public Health*,2017:14:632.
30. Huertas-Perez JF, Arroyo-Manzanares N, Garcia-Campana AM, Gamiz-Gracia L. Solid phase extraction as sample treatment for the determination of Ochratoxin A in foods: A review. Crit. Rev. Food Sci. Nutr,2017:57:3405-3420.
31. Pereira VL, Fernandes JO, Cunha SC. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. Trends Food Sci. Technol,2014:36:96-136.
32. Le VT, Vasseghian Y, Dragoi EN, Moradi M. Mousavi Khaneghah A. A review on graphene-based electrochemical sensor for mycotoxins detection. Food Chem. Toxicol,2021:148:111931.
33. Turner NW, Bramhmbhatt H, Szabo-Vezse M. Poma A, Coker R, Piletsky SA. Analytical methods for determination of mycotoxins: An update Anal. Chim. Acta,2015:901:12-33.
34. Lattanzio VMT, Von Holst C Lippolis V, De Girolamo A, Logrieco AF, Mol HGJ, Pascale M. Evaluation of Mycotoxin Screening Tests in a Verification Study Involving First Time Users. Toxins,2019:11:129.
35. Wolf K, Schweigert JF. Mycotoxin Analysis: A Focus on Rapid Methods; Partnership for Aflatoxin Control in Africa: Addis Ababa, Ethiopia, 2018.
36. Urusov AE, Zherdev AV. Dzantiev BB. Immunochemical methods of mycotoxin analysis (review). Appl. Biochem. Microbiol,2010:46:253-266.
37. Hendrickson OD, Chertovich JO, Zherdev AV, Sveshnikov PG, Dzantiev BB. Ultrasensitive magnetic ELISA of zearalenone with pre-concentration and chemiluminescent detection. Food Control,2018:84:330-338.
38. Younis M, Younis A, Xia XH. Use of Biosensors for Mycotoxins Analysis in Food Stuff. In Nanobiosensors: From Design to Applications; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany,2020:171-201.
39. Evtugyn GA, Shamagsumova RV, Hianik T. 2-Biosensors for detection mycotoxins and pathogenic bacteria in food. In Nanobiosensors; Grumezescu, A.M., Ed.; Academic Press: Cambridge, MA, USA, 2017, 35-92.
40. Yang J, Li J, Jiang Y, Duan X, Qu H, Yang B, *et al*. Natural Occurrence, Analysis, and Prevention of Mycotoxins in Fruits and their Processed Products. Crit. Rev. Food Sci. Nutr,2014:54:64-83.
41. Wacoo AP, Wendiro D, Vuzi PC, Hawumba JF. Methods for Detection of Aflatoxins in Agricultural Food Crops. J. Appl. Chem, 2014, 706291.
42. Odhav B, Naicker V. Mycotoxins in South African traditionally brewed beers. Food Addit. Contam,2002:19:55-61.
43. Abrunhosa L, Paterson RRM, Kozakiewicz Z, Lima N, Venancio A. Mycotoxin production from fungi isolated from grapes. Lett. Appl. Microbiol,2001:32:240-242.
44. Caldas ED, Silva ACS. Mycotoxins in corn-based food products consumed in Brazil: An exposure assessment

for fumonisins. J. Agric. Food Chem,2007:55:7974-7980.
45. Rizzo I, Vedoya G, Maurutto S, Haidukowski M, Varsavsky E. Assessment of toxigenic fungi on Argentinean medicinal herbs. Microbiol. Res,2004:159:113-120.
46. Singh J, Mehta A. Rapid and sensitive detection of mycotoxins by advanced and emerging analytical methods: A review. Food Sci. Nutr,2020:8:2183-2204.
47. Yang Y, Li G, Wu D, Liu J, Li X, Luo P, *et al*. Recent advances on toxicity and determination methods of mycotoxins in foodstuffs. Trends Food Sci. Technol,2020:96:233-252.
48. Pascale M, De Girolamo A, Lippolis V, Stroka J, Mol HGJ, Lattanzio VMT. Performance Evaluation of LC-MS Methods for Multimycotoxin Determination. J. AOAC Int,2019:102:1708-1720.
49. Rodríguez-Carrasco Y, Molto JC, Berrada H, Manes J. A survey of trichothecenes, zearalenone and patulin in milled grain-based products using GC–MS/MS. Food Chem,2014:46:212-219.
50. Al-Jaal B, Salama S, Al-Qasmi N, Jaganjac M. Mycotoxin contamination of food and feed in the Gulf Cooperation Council countries and its detection. Toxicon,2019:171:43-50.
51. Liang Y, Huang X, Chen X, Zhang W, Ping G, Xiong Y. Plasmonic ELISA for naked-eye detection of ochratoxin A based on the tyramine-H2O2 amplification system. Sens. Actuators B Chem,2018:259:162-169.
52. Oplatowska-Stachowiak M, Reiring C, Sajic N, Haasnoot W, Brabet C, Campbell K, *et al*. Development and in-house validation of a rapid and simple to use ELISA for the detection and measurement of the mycotoxin sterigmatocystin. Anal. Bioanal. Chem,2018:410:3017-3023.