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Chapter

Review of QuEChERS Methods for the Analysis of Mycotoxins in Food Samples

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Abstract

Mycotoxins are secondary metabolites present in foods which can cause adverse effects on humans and animals. Therefore, developing a simple, effective, sensitive and validated analytical method to monitor mycotoxins is essential. Sample preparation is an important step in the analysis of mycotoxins and other contaminants from complex food matrices. Food industries in developed and developing countries have faced serious challenges with contamination of mycotoxins especially aflatoxin in food and feed products. Thus, corn and cereal-based foods are mostly affected right from pre and postharvest periods. Owing to the complexity and structural nature of mycotoxins in foods and feeds there is an urgent need for simple, effective and environmentally friendly methods of sample preparation for the detection and quantification of aflatoxins in food samples. The paper reviews the application of the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method for the analysis of aflatoxins in foods.

Keywords: aflatoxins, chromatographic analysis, GC/MS, LC/MS, QuEChERS, sample preparation

1. Introduction

Aflatoxins are a group of chemically similar poisonous, carcinogenic fungal secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, which are abundant in warm and humid regions of the world. They are probably the most intensively researched toxins in the world due to their carcinogenic and mutagenic effects. Aflatoxins have also been identified as a potential biological weapon for food and water contamination. The word aflatoxins is the combination of three words: first letter "A" from genus *Aspergillus*, next three letters "FLA" from species *flavus*, and the noun "TOXIN". Aflatoxins are quite stable and are resistant to degradation [1, 2]. There are about 18 different aflatoxins, and six types have been identified to be more important and they are labeled AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2, and they

exhibit different molecular structures. The B-group have cyclopentane ring and exhibits blue fluorescence under UV light, while the G group contains lactone ring and exhibits yellow-green fluorescence under UV light. Aflatoxins M1 and M2 are hydroxylated derivatives of aflatoxins B and were first isolated from milk. The behavior under UV light made them easily identified and quantified using fluorescence spectroscopy [1, 3]. The aim of this work is to review various aspects of QuEChERS techniques including its various modification for the analysis of aflatoxins in food samples.

2. Aflatoxin and its health impacts

Food industries in developed and developing countries are facing serious challenges with contamination of mycotoxins especially aflatoxin in food and feed products. The Food and Agricultural Organization (FAO) concluded that around 25% of the world's cereals are contaminated by mycotoxins including aflatoxins [4]. The most common food commodities affected by aflatoxins are cereals (corn, wheat, barley, maize, oats and rye), nuts (hazelnut, peanut and pistachio nut), dried fruits (fig), and spices (chili powder) [2]. Thus, aflatoxins are quite chemically stable and are highly resistant to degradation. Among the 18 common groups of aflatoxins, B1, B2, G1, G2, M1 and M2 are the major classes and derivatives of bifuranocoumarins. Health implications of contaminated aflatoxins in humans and animals through consumption, contact or inhalation of foodstuffs in both developed and developing countries cannot be underestimated, where billions of people are chemically exposed to uncontrolled amounts of aflatoxins, which causes disease known as aflatoxicosis [1]. Aflatoxins are toxic and fatal in poultry animals (livestock) and are carcinogenic to humans [5].

The International Agency for Research on Cancer (IARC) classified AFBI as class I human carcinogen and has a positive association between dietary aflatoxins and liver cell cancer (LCC). This was the third leading cancer death globally. Vomiting, abdominal pain, pulmonary edema, convulsions and coma. Enlargement of internal organs such as liver, kidneys and heart are common symptoms of aflatoxicosis. Different regions and countries have set maximum levels (MLs) for different mycotoxins in food. In Europe, limits of 2 ppb (for aflatoxin B1) and 4 ppb (for total aflatoxins (B1 + B2 + G1 + G2), for cereals and cereal products (including maize and maize products) for direct human consumption are in place. Likewise, MLs of 5 ppb for aflatoxin B1 and 10 ppb for total aflatoxins are set for maize to be sorted or otherwise processed physically before human consumption. The European Commission further set a method for sampling cereals and cereals products in view of the prescribed limits. The regulated limits of mycotoxins in the European region are defined in the regulation of the European Community EG-VO 1881/2006. Codex Alimentarius Commission is responsible for setting maximum limits for mycotoxins in food and feed at the global level. The Codex Commission has already adopted MLs for mycotoxins as shown below [6]:

1. A maximum level of 10 ppb for total aflatoxins in tree nuts (almonds, hazelnuts, pistachios and shelled Brazil nuts) 'ready-to-eat'.

2. ML of 15 ppb for total aflatoxins in peanuts and tree nuts destined for further processing.

3. ML of 2000 ppb for fumonisins in maize and maize flour for direct human consumption.

4. ML of 4000 ppb for fumonisins in maize for further processing.

5. ML of 2000 ppb for deoxynivalenol in raw cereal grains (wheat, maize and barley).

6. ML of 1000 ppb for deoxynivalenol in flour, semolina, meal and flakes derived from wheat, maize and barley.

7. ML of 200 ppb for deoxynivalenol in cereal-based foods for infants and young children.

3. Sampling in Aflatoxins

Aflatoxin is a subclass of mycotoxins which are strains of the fungi *Aspergilllus flavus* and *A. parasiticus* and the less common *A. nomius*. Aflatoxins B1, B2, G1, G2, M1 and M2 are the most common types of Aflatoxins, which can be grouped in two based on their chemical structure, that is difurocoumarocyclopentanone and ifurocoumarolactone [2]. However, many foods and feeds that are prone to mycotoxin contamination cannot be directly analyzed in the absence of extraction and clean-up steps [7]. Researchers have used various extraction and clean-up methods to extract aflatoxins from complex matrices [7]. Dry, wet and cryogenic grindings are common homogenization techniques in cereal-based foods, oil seeds, spices, trees nuts and peanuts, contaminated by aflatoxins. Spanjer et al. [8] successfully used dry milling to process peanut, pistachio, wheat, maize, cornflakes, raising and figs for the analysis of different mycotoxins including aflatoxins. Evaluation of homogenization is always done in terms of analytical results, coefficients of variation for different mills, sample and subsample sizes and particle size distributions [7].

The European Union defined sampling method for mycotoxins in agricultural commodities through Commission Regulation No EC401/2006, to show that sample preparation plays important role in the precision of the determination of mycotoxins. Hydrophobic mycotoxins are extracted in the presence of organic solvents, such as methanol, acetone, chloroform and acetonitrile, while polar mycotoxins are extracted in mixture of organic solvents and water [3, 9]. Studies have shown that near infrared region (NIR) (800–2500 nm) is capable of differentiating kernels containing >100 ppb or <10 ppb levels of total aflatoxins. Research conducted on 168 samples of corn collected from different parts of Italy demonstrates that FT-NIR spectroscopy is better, easier and faster to detect FB1 and FB2 in corn compared to other analytical methods such as HPLC and ELISA [10].

3.1 Sample Preparation

Sample preparation stage is the most crucial and critical step in the analysis of contaminants in complex food samples [11]. Owing to the complexity and structural nature of mycotoxins in foods and feeds there is an urgent need for simple, effective and environmentally friendly methods of sample preparation for the detection and

quantification of aflatoxins in food samples [3]. The goals of sample treatment step are as follows.

i. ability to use smaller amount of sample

ii. improvement in online methods and reduce manual operations

iii. the usage of no or small volumes of organic solvent with less waste and friendly environment in order to approache green chemistry [12].

Indeed, sample preparation is of great importance in analytical procedures because its steps account for one-third of the errors generated by analytical [13]. An efficient sample preparation method provides reliable, precise and accurate results, especially when trace or ultra-trace level of analytes in complex matrices (biological and environmental) are analyzed. Low operational cost, adequate removal of matrices interference, use of small amount of solvent, limiting the number of steps and high reproducibility and recovery, high sample throughput are characteristics of good sample preparation [14].

Extraction methods based on QuEChERS (quick, easy, cheap, effective, rugged and safe) developed by Anastassiades and his co-researchers. [15] have been widely used in analysis of mycotoxins (aflatoxin, ochratoxin A, zearalenone, fusarenon X, α and β zearalenone) due to their simplicity and effectiveness for isolating mycotoxins from complex matrices. In contrast, traditional methods of extraction such as liquid–liquid extraction and solid phase extraction use highly toxic solvents, time-consuming and large amount of sample. QuEChERS ensures minimum sample loss by limiting the number of steps, improving sample throughput, low operational cost and effective removal of matrix component interference with high productivity and recovery [14].

The extraction method (**Figure 1**) is based on microscale extraction/partitioning followed by dispersive solid phase extraction (dSPE) for cleanup [16]. The analyte is partitioned between an aqueous and an organic layer by using $MgSO_4$ and NaCl, followed by manual mixing and then centrifugation for a period of time and then the

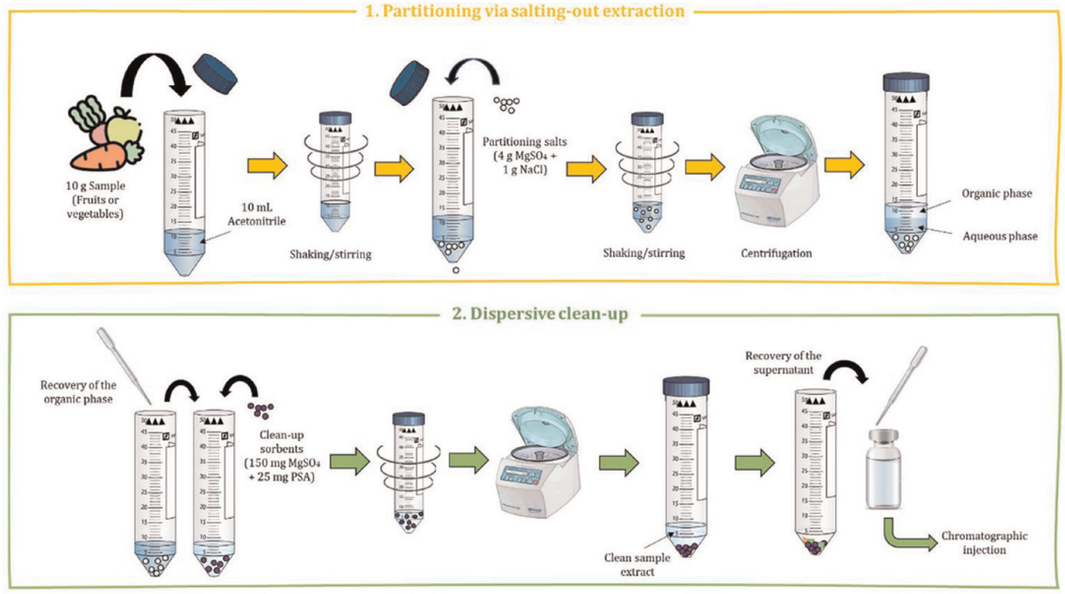


Figure 1.
Steps in original QuEChERS extraction procedure [19].

supernatant is cleaned up with the combination of primary-secondary amine (PSA) adsorbent and $MgSO_4$ for the removal of interfering substances [17, 18]. The aliquot of the cleaned-up extract can then be analyzed with any of the analytical instruments.

4. Application of QuEChERS in extraction of aflatoxins

In recent times, researchers have applied QuEChERS for the analysis of aflatoxins in different food samples, although, it was initially developed for the analysis of pesticide residues [15] in fruit and vegetable samples. The different modifications of QuEChERS methods as employed in the determination of aflatoxins in food samples are hereby discussed.

Sirhan et al. [2] used QuEChERS-HPLC to detect aflatoxin in 609 samples of food consisting of 274 cereals, 87 peanuts, 78 peanut butter, 46 nuts, 46 sesame seeds; 61 Pistachio nuts, 51 seeds (sunflower, watermelon) and 51 green coffee. About 1−4 kg of each representative sample was collected and kept in dark room at 20−25°C (room temperature). A fine and homogenous powdered material was obtained through grinding and mixing processes. Factors such as solvent extraction, type and amount of drying agent, the extraction time and solvent sample ratio were optimized. All experiments were carried out using the same procedure and were tested in a blank peanut sample that had been spiked with 10.0 μg/l of aflatoxin B1 and G1 and 3.0 μg/l of aflatoxin B2 and G2. The linearity, accuracy, limit of defection (LOD), limit of quantification (LOQ), intra-day precision and inter-day precision were validated in this study. The linear concentration range from 0.059 to 30 μg/kg for aflatoxin B2 and G2 and from 0.195 to 100 mg/kg for aflatoxin B1 and G1, with correlation coefficient greater than 0.993 for all the targeted analytes The limit of detection (LOD) and limit of quantitation (LOQ) were found respectively to be 0.17 and 0.57 μg/kg in B1, 0.05 and 0.18 μg/kg in B2, 0.35 and 1.17 μg/kg in G1, and 0.06 and 0.20 μg/kg in G2. The recoveries obtained ranged between 76.3 and 98.0% with RSD values of less than 10%. The sensitivity of the method was estimated by the LOD and LOQ [2].

AFBI and AFM1 in 40 milk samples were determined simultaneously using QuEChERS with ultrahigh performance liquid chromatography coupled to quadrupole orbitrap mass spectrometry [20]. A modified QuEChERS was used to extract aflatoxin in milk. An aliquot of 10 ml sample was transferred into falcon tube containing 2.5 ml distilled water and 5.0 ml acetonitrile containing 3.35% of formic acid was then added. The mixture was vigorously vortexed for 2 min before it was subjected to ultrasonic extraction for 15 min. This was followed by addition of 4.0 g of anhydrous Na_2SO_4 and 1.2 g of NaCl, and the tube was shaken by hand for 2 min and then centrifuged for 3 min at 4000 rpm. Consequently, the supernatant was reconstituted with 500 μl of mixture of MeOH:H_2O (70:30, v/v) and filtered, then transferred for UHPLC-Q-Orbitrap HRMS analysis.

The validation and evaluation of method developed by Rodriguez-Carrasco and co-researchers were performed in accordance with SANCO (2011), by determining the linearity, matrix effect, precision, specificity and sensitivity. The recovery ranged from 75 to 91% and 81–96% for AFM1 and AFB1, respectively with RSD ranging from 7 to 16%. The linearity was found between 0.002 and 20 μg/l, with correlation coefficient R^2 greater than 0.9990. The matrix effect which was expressed as a ratio percentage between the slope of the matrix-matched calibration curve and the curve in solvent was 72 and 65% for AFB1 and AFM1, respectively. The LOQ was found to be

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (µg/kg) (R^2)	Recovery (%) (RSD %)	LOD (µg/kg)	LOQ (µg/kg)	Reference
AF(B1, B2, G1, G2)	Cereals, peanuts, peanut butter, nuts, sesame seeds, pistachio nuts, green coffee	1 g sample, 3 ml, ACN/H_2O, (40/60, v/v%), 1.32 g $MgSO_4$, 0.25 g NaCl	Filter through 0.45 µm Nylon syringe filter	HPLC-FLD	0.059−100 (>0.993)	76.3−98.0 (<10)	0.06–0.35	0.18–1.17	[2]
B1, M1	Milk	10 ml sample 4.0 g Na_2SO_4, 1.2 g NaCl, 2.5 ml H_2O, 5.0 ml ACN (3.35% HCOOH)	Supernatant + MeOH: H2O (70:30 v/v) then filtered	HPLC-Q-orbitrap MS	0.002−20 µg/l (>0.999)	75–96 (7–16)	0.001	−0.002	[20]
AF(B1, B2, G1, G2), FFB1 B2), DON, OTA (ZON)	Brown rice	1 g sample, 2.0 g $MgSO_4$, 0.50 g NaCl, 0.50 g sodium citrate tribasic dihydrate and 0.25 g sodium citrate dibasic sesquihydrate 5 ml 10% (v/v) HOAc in ACN	300 mg $MgSO_4$, 50 mg C18, 25 mg PSA and 25 mg silica	UHPLC–MS/MS	5.0–1000 (n.r)	81–101 (5–19)	1.4–2.5	4.1−8.5	[29]
AF(B1, B2, G1, G2)	Rice	3.3 g sample 6.6 ml H_2O, 10 ml ACN, 4 g $MgSO_4$, 1 g NaCl	150 mg PSA. 600 mg $MgSO_4$	HPLC-FLD	4−40 (>0.999)	102−128 (<12)	0.05−6.0	0.15−8.0	[21]
11 mycotoxins	Plant-based beverages	50 ml sample 4 g $MgSO_4$, 1 g NaCl, 10 ml ACN(1% HCOOH)	Supernatant filtered through 0.2 µm nylon filter	UHPLC–MS/MS	0.05−2000 µg/l	80–91 (n.r)	0.02−0.4 µg/l	0.05–15.0 µg/l	[23]
15 mycotoxins		10 g sample, 40 ml 84% ACN/H_2O	Supernatant evaporated and reconstituted with	HPLC–MS/MS	0.5−400 (n.r)	80.1−95.5 (10.5–19.6)	0.70−5.0	n.r	[22]

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (μg/kg) (R^2)	Recovery (%) (RSD %)	LOD (μg/kg)	LOQ (μg/kg)	Reference
			ethyl acetate and cyclohexane (50:50 v/v) and filtered through 0.45 μm nylon filter						
13 mycotoxins	plums, raisins, apricots, figs and dates	5 g sample, 7.5 ml H_2O, 1% HOAc, 22.5 ml ACN, 7.5 g $MgSO_4$, 3 g NaCl	Supernatant redissolved in 1 ml ammoniom formate/ methanol +1% HOAc, filtered through 0.22 μm filter	LC–MS/MS	n.r (>0.998)	60–135 (≤ 20)	0.08–15	0.2–45	[24]
16 mycotoxins	almonds, peanuts, walnuts, hazelnuts, pecan nuts, cashews, Brazil nut, pine nuts	1 g sample, 5 ml H_2O, 5 ml ACN (5% HCOOH) 2 g $MgSO_4$, 0.5 g NaCl	50 mg C18, 50 mg Z-sep$^+$,	LC-MS/MS	11.25−500 (>0.970)	70−93 (≤13)	0.4−3.5	1.25−5	[25]
AF(B1, B2, G1, G2)	Honeybee	10 g sample, 15 ml ACN, 5 ml H_2O, 6 g $MgSO_4$, 1.5 g NaCl	100 mg PSA, 600 mg $MgSO_4$	HPLC-UV-DAD	n.r	88.25−92.9	n.r	n.r	[26]
AF(B1, G1)	maize	10 g sample, 1.67 g NaOAc, 10 ACN (1% HOAc), 4 g $MgSO_4$	No clean-up	HPLC-FLD	0.4−20 μg/l (>0.99)	79.5−99.73 (1.10−2.27)	0.08−16	n.r	[27]
AF(B1, B2, G1, G2, M1, M2)	Peanut	5 g sample, 10 ml H_2O, 10 ml hexane, 15 ml ACN), 4 g $MgSO_4$, 1,5 g NaCl	Supernatant dried +2 ml MeOH/H_2O and filtered	UHPLC–MS/MS	0.15–15 μg/l (>0.99)	71.3–100.3 (1.5−12.4)	0.03−0.26	0.10–0.88	[37]

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (µg/kg) (R^2)	Recovery (%) (RSD %)	LOD (µg/kg)	LOQ (µg/kg)	Reference
AF(B1, B2, G1, G2)	Wheat and wheat by-products	2 g sample, 10 ml MeOH/H_2O/ACN, 1.5 g $MgSO_4$, 0.5 g NaCl	Filtered through a 0.45 µm	HPLC-FD	1.2−24 (>0.99)	70−110 (>15)	0.6	1.2	[33]
12 mycotoxins	Maize, wheat, black pepper, coffee	5 g sample, 10 ml H_2O, 10 ml ACN, (20% HOAc), 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate tribasic dehydrate	900 mg $MgSO_4$, 150 mg PSA	UHPLC–MS/MS	0.8−2000 µg/l (>0.99)	60−120 (0.026–36.7)	n.r	n.r	[34]
AFB1	Rice, peanut, corn, fishmeal	10 g sample, 20 ml ACN/MeOH (40/60%), 4 g $MgSO_4$, 1 g NaCl	No clean up step	HPLC-FLD	5−100 (>0.98)	82.50–109.85 (0.57–11)	0.2−1.2	0.3−1.5	[35]
AF(B1, M1)	Milk, dairy products	5 g sample, 5 ml H_2O, 1 g NaCl, 1 g trisodium citrate dehydrate, 0.5 g disodium hydrogen citrate sesquihydrate	950 mg MgSO4, 200 mg Bondesil PSA, 200–400 mg C18	HPLC-FLD	0.03−10	51.2−75.7 (n.r)	0.01−0.1	0.03–0.3	[36]
AF(B1, B2, G1, G2, M1, M2)	Milk	1.5 g sample, 10 ml H_2O, 10 ml Hexane, 15 ml ACN 1% HOAc), 6 g $MgSO_4$, 1.5 g NaCl	Supernatant dried +5 ml MeOH/H_2O, filtered through a 0.22 µm polyethylene filter	UHPLC–MS/MS	1. – 1.5 µg/l (≥ 0.99)	72.8–121 (0.7–16.7)	0.017–1.45	0.005–0.44	[28]
17 mycotoxins	Cereal	5 g sample, 10 ml H_2O, 10 ml ACN (0.5% HOAc), 4 g $MgSO_4$, 1 g NaCl,	Evaporated to dryness and reconstituted with 75 µl methanol	LC–MS/MS	0−10,000 (>0.98)	73−130 (0–18)	n.r	0.5−100	[38]

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (μg/kg) (R^2)	Recovery (%) (RSD %)	LOD (μg/kg)	LOQ (μg/kg)	Reference
AFM1	Milk	10 ml sample, 10 ml ACN, 1 g NaCl, 4 g $MgSO_4$	150 mg $MgSO_4$, 50 mg PSA, 50 mg C18.	LC–MS/MS	0–1.0	85–97 (14.5–16.3)	0.02	0.4	[39]
13 mycotoxins	Feedstuff	5 g sample, 10 ml H_2O (10% HCOOH, 10 ml ACN, 1 g NaCl, 4 g $MgSO_4$	25 mg PSA, 25 mg C18	LC–MS/MS	0.5–500 μg/l (>0.98)	70.1–115.6 (0.1–11.3	0.8333–16.7 μg/l	2.5–50 μg/l	[40]
16 mycotoxins	Vegetable oil	1 g sample, 2 ml H_2O, 18 ml ACN, 4 g Na_2SO_4, 1 g NaCl	100 mg C18	HPLC-MS	0.2–500 (>0.99)	72.8–105.8 (0.2–6.3)	0.04–2.9	0.12–1-	[41]
OTA	Cereals	1 g sample, 3 ml H_2O/ ACN/HOAc, 0.8 g $MgSO_4$, 0.2 g NaCl	Filtered through a 0.45 μm nylon syringe filter	HPLC-FLD	3.75–120 μg/l (>0.99)	85.2–109.8 (<12)	0.18 to 0.62	0.60–2.08	[42]
20 mycotoxins	Grains	5 g sample, 25 ml ACN/H_2O (1% HOAc), 4 g $MgSO_4$, 1 g NaCl, 1 g of $Na_3Cit{\cdot}2H_2O$, and 0.5 g of $Na_2Cit{\cdot}1.5H_2O$	20 mg Fe_3O_4-MWCNTs, then filtered	UHPLC–MS/MS	1–500 (>0.99)	73.5–112.9 (1.3–12.7)	0.0006–1.6337	0.0021–5.4457	[43]
AF(B1, B2, G1, G2), OTA	Apple juice, raisin, wheat flour, peanut, spices	2–2.5 g sample, 10 ml ACN (1% HOAc), 7.5 ml of H_2O, 4 g $MgSO_4$, 1 g NaCl, 1 g of $Na_3Cit{\cdot}2H_2O$, and 0.5 g of $Na_2Cit{\cdot}1.5H_2O$	0.2 g PSA, 0.6 g $MgSO_4$.(low fat) 150 mg of C18, 900 mg $MgSO_4$ (high fat)	LC–MS/MS	1–30 (>0.996)	81.94–101.67 (0.12–10.28)	0.05–0.1	0.08–0.3	[44]
ZEA, T-2, AFB1, DON, OTA	Corn flour	5 g sample, 15 ml ACN/H_2O/HCOOH, 5 g $MgSO_4$, 1 g NaCl,	1 g $MgSO_4$ and 0.3 g PSA, dried +1 ml MeOH	LC–MS/MS	2–1800	92.9–103.8 (3.7–20)	2–75	0.6–25	[45]

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (μg/kg) (R^2)	Recovery (%) (RSD %)	LOD (μg/kg)	LOQ (μg/kg)	Reference
AF(B1, B2, G1, G2), T-2, HT-2	Cereal derived products	2 g sample, 10 ml H_2O (1% HOAC), 10 ml ACN, 4 g $MgSO_4$, 1 g NaCl,	2 ml extract evaporated +200 μl MeOH/H_2O, then filtered	LC–MS/MS	2–140 (>0.990)	83.8–102.9 (14.3–15.7)	0.5–100	1–200	[46]
ZEA, DON	Cereal	4 g sample, 16 ml ACN/H_2O, 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g sodium citrate dehydrate, 1 g sodium citrate sesquihydrate	300 mg PSA, 100 mg $MgSO_4$, dried +1 ml of MeOH/PBS	ELISA	13.64–983.52 ng/ml ((n.r)		2.58–17.31 ng/ml	n.r	[47]
AF(B1, B2, G1, G2, M1, M2), OTA, OTB, ZEA	Egg	5 g sample, 5 ml Na_2EDTA, 20 ml of ACN (1% of HOAc), 4 g Na_2SO_4, 1 g NaCl	15 mg of Fe_3O_4-MWCNTs, separated by external magnetic field. 1 ml extract +1 ml MeOH/H_2O, filtered	UHPLC–MS/MS	1–100 (n.r)	71.8–100.0 (1.6–17.3)	n.r	0.2–11.8	[48]
26 mycotoxins	Sesame butter	2.5 g sample, 20 ml ACN/H_2O (0.1% HCOOH), 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, 0.5 g sodium hydrogen citrate sesquihydrate	150 mg C18, 900 mg $MgSO_4$,	UHPLC–MS/MS	0.5–500 ng/ml (>0.994)	48.70–111.70	0.05–7.25	0.11–21.74	[49]
AF(B1, B2, G1, G2), F (B1, B2, B3), ZEA, DON	Corn	2 g sample, 20 ml ACN/H_2O, 2 g $MgSO_4$, 0.5 g NaCl	30 mg C18	UPLC-Q-TOFMS	2.5–2000 (>0.991)	68.0–120.0 (0.18–6.29)	0.05–50	0.1–200	[50]

Aflatoxins	Sample Matrix	QuEChERS procedure	Clean-Up method	Instrumental analysis	Linearity (μg/kg) (R^2)	Recovery (%) (RSD %)	LOD (μg/kg)	LOQ (μg/kg)	Reference
14 mycotoxins	Rice	10 g sample, 4 g $MgSO_4$, 1 g NaCl 4 g, 1 g sodium citrate tribasic dihydrate and 0.5 g sodium citrate dibasic sesquihydrate	1.2 g $MgSO_4$, 0.25 g C18, 0.25 g Al-N	UHPLC–MS/MS	10–2500 (>0.99)	70–98.5 (<7.0)	0.5–15	1.7–50	[52]

Key: *RSD, relative standard deviation;* R^2*, correlation coefficient; n.r, not reported; LOQ, limit of quantitation; LOD, limit of detection; AF, aflatoxin, DON, deoxynivalenol; F, Fumonisin; OTA. Ochratoxin A, OTB, ochratoxin; ZEA, zearalenone; T-2, T-2 toxin; HT-2, HT-2 toxin; MeOH, methanol; ACN, acetonitrile, HCOOH, formic acid; HOAc, acetic acid; HPLC, high performance liquid chromatography; UHPLC, ultra-high performance liquid chromatography; MS, mass spectrometry; FLD, fluorescence detector; UV, ultraviolet; DAD, diode array detector; Q-TOF, quadruple time-of-flight; PSA, primary secondary amine; Al-N, aluminum nitride.*

Table 1.
Applications of QuEChERS techniques for the analysis of aflatoxins in food samples.

0.001 μg/kg and LOQ of 0.002 μg/kg, and it showed that the developed method is suitable for the determination of trace amount of aflatoxins in milk samples.

Rice samples belonging to different varieties were purchased for aflatoxins detection [21]. A modified QuEChERS method was used for the extraction of the aflatoxin from the sample. An aliquot of 3.3 g of homogenized rice was measured into a 50 ml Teflon centrifuge tube then aflatoxins were spiked at 6, 12 and 20 μg/kg concentrations. Prior to QuEChERS extraction, the spiked homogenates were stored in the dark at room temperature for 6 h to enhance absorption of aflatoxin into the sample matrix. Water (6.6 ml) and acetonitrile (10 ml) were added at 3 min intervals followed by vigorous shaking to obtain a homogenous mixture. Subsequently, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added, shaken and centrifuged at 4000 rpm and for 5 min. The supernatant (5 ml) was transferred into another centrifuge tube containing 150 mg of PSA and 600 mg of $MgSO_4$. The mixture was shaken for 1 min and centrifuged for 5 min at 4000 rpm. The supernatant was injected into the HPLC (mobile phase containing water/methanol/acetonitrile mixture (65:25:10, v/v/v%) pumped at isocratic mode at rate of 1 ml/min, at injection volume of 20 μl. The detection was achieved at excitation and emission wavelengths of 360 and 450 nm, respectively. Validation of methods showed the limits of detection and quantification were ≤6 and ≤8 μg/kg, respectively. The linearity was between 6 and 20 μk/kg with a correlation coefficient greater than 0.99. The intra-day and inter-day recoveries were in the range 104–119% and 104–113% with RSD ≤ 12% for concentrations between 6 and 20 μg/kg.

A method, whichh was found to be sensitive, reliable, and selective was developed for the determination of 15 mycotoxins in foods and feeds using HPLC-MS with gel permeation chromatography combined with QuEChERS purification [22]. For the sample preparation, 10 g of each homogenized sample was transferred into a 100 ml centrifuge tube followed by addition of 40 ml of 84% (v/v) acetonitrile/water mixture and the mixture was homogenized for 3 min with high-speed homogenizer. The mixture was then centrifuged at 10,000 rpm for 10 min and 16 ml of the supernatant was evaporated to dryness under a stream of nitrogen at 5°C. The residue was then redissolved with 8 ml of mixture of ethyl acetate and cyclohexane (50:50 v/v) and filtered through 0.45 μm nylon filter for gel permeation chromatography (GPC) injection. A 50:50 v/v% of ethyl acetate/cyclohexane was used as the GPC mobile phase at a flow rate of 4.7 ml/min. The eluent of the GPC was collected and evaporated to dryness using rotary evaporator and the residue was redissolved with 2.5 ml acetonitrile. The redissolved residue was then vortexed with 150 mg of octadecylsilane for 1 min and an aliquot of 2 ml of the supernatant was transferred into a test tube and dried by stream of nitrogen at 50°C. The residue was then redissolved in 1 ml of methanol/10 mmol/l ammonium acetate (1:1 v/v). Finally, the solution was filtered through a 0.22 μm nylon filter and was subjected to HPLC operated at a column temperature of 35°C, with injection volume of 20 μl and mobile phase made up of solvent A (10 mmol/l ammonium acetate used for the ESI+ mode and 0.1% (v/v) aqueous ammonia used for the ESI- mode) and solvent B (methanol). The LOD of the 15 mycotoxins ranged from 0.70−5.0 μg/kg, and the recoveries ranged from 80.1−95.5% with relative standard deviation between 10.5 and 19.6%. The method gave good linear relationships and good coefficients of determination ($r^2 > 0.996$) were achieved over the concentration range of 0.5–400 ng/ml.

Miro-Abella et al. [23] used QuEChERS method followed by liquid chromatography–tandem mass spectrometry to determine 11 mycotoxins in plant-based beverages which were reported to yield 80–91% recoveries with better repeatability and reproducibility values. Limit of quantification was between 0.05 μg/l (for AFGI and AFBI)

and 15 μg/l for decoxynivalenol and fumonisin B2. For the preparation of samples using QuEChERS, 50 ml centrifuge tube containing mixture of 10 ml of sample, 10 ml acetonitrile containing 1% formic acid was shaken for 1 min, then, 4 g of $MgSO_4$ and 1 g of NaCl were added to the solution and shaken vigorously for 3 min. The tubes were later centrifuged at 10,000 rpm at 20°C for 5 min. This was followed by diluting 1 ml of aliquot of organic layer as supernatant (v/v) with solvent A (water) of the mobile phase and filtered with 0.2 μm nylon filter. The linearity of the method was better with $r^2 \geq 0.993$ in all matrices and LODs were 0.001 μg/l (for AFG2, AFG1, AFB2 and AFB1), 0.04 μg/l (for FB1, FB2 and ZEA), 0.01 μg/l (for OTA and T-2), 0.1 μg/l (for DON) and 0.25 μg/l (for HT-2), with LOQs of 0.003 μg/l (for AFG2, AFG1, AFB2 and AFB1), 0.2 μg/l (for FB1, FB2 and ZEA), 0.03 μg/l (for OTA and T-2), 0.3 μg/l (for DON) and 0.9 μg/l (for HT-2). Linear range was from LOQ to 100 μg/l (for AFG2, AFG1, AFB2, AFB1 and OTA), to 500 μg/l (for DON, FB2 and T-2) and to 1000 μg/l 1 (for FB1, HT-2 and ZEA). The results of the developed method showed that QuEChERS approach was suitable for the extraction of the target mycotoxins from different food and feed matrices.

A method for the analysis of mycotoxins in dried fruits, such as plums, raisins, apricots, figs and dates was developed using a modified QuEChERS procedure with LC–MS/MS analysis. Thirteen different mycotoxins were investigated in the fruit samples. The method developed involves homogenizing 5 g of sample with7.5 ml of water containing 1% acetic acid for 3 min. And the mixture obtained was then extracted with 22.5 ml of acetonitrile for 3 min with a vortex. This was followed by the addition of 7.5 g of $MgSO_4$ and 3 g of NaCl and the mixture was shaken manually for 1 hr. The mixture was centrifuged for 10 min at 5000 rpm and the supernatant was collected and evaporated to dryness and was then redissolved with 1 ml of 5 mM aqueous ammonium formate/methanol solution acidified with 1% acetic acid. The resulting solution was filtered through 0.22 μm PTFE filter prior to LC/MS/MS analysis. The limit of detection (LOD) was found to be 0.08–15 μg/kg, limits of quantification (LOQ) was between 0.2–45 μg/kg and recovery in the spiked sample ranged from 60 to 135% with RSD ≤ 20 except in beauvericin. Thus, values were below an acceptable limit set by the European Union for the legislated mycotoxins [24].

The occurrence of 16 mycotoxins belonging to different chemical classes was assessed in several nut products using QuEChERS followed by LC–MS/MS analysis. The use of different clean-up sorbents was extensively evaluated. The samples (50 g) were grinded and an aliquot of 1 g of the homogenized sample was transferred into 60 ml centrifuge tube, followed by addition of 5 ml of water and 50 μl of internal standard. Exactly 5 ml of acetonitrile containing 5% formic acid, 2 g of $MgSO_4$ and 0.5 g of NaCl were added and then shaken vigorously by hand for 2 min and centrifuged for 5 min at 3750 rpm. The supernatant (1 ml) was then transferred to the dSPE clean-up tube containing 50 mg of C18 and 50 mg of Z-sep + and centrifuged for 3 min at 1750 rpm. The upper layer was evaporated to dryness under a gentle stream of nitrogen and the dry extract was reconstituted in 250 μl of mixture of methanol/water/acetic acid (97:2:1, v/v) containing 5 mM of ammonium acetate. The method validated using an internal standard calibration method gave linearity between 1.25–500 μg/kg and the detection limits achieved between 0.4–3.5 μg/kg and LOQ ranged from 1.25 to 5 μg/kg for the targeted analytes. The average recoveries ranged between 70 to 93% with RSD ≤ 13%. Eleven out of the 16 mycotoxins were found in 37 nut samples, with highest contamination found in cashew sample containing 336.5 μg/kg of deoxynivalenol (DON) [25].

Aflatoxins B1, B2, G1 and G2 and carbamate pesticide contamination were evaluated in 44 samples of bee honey locally produced in Egypt and 9 other countries using QuEChERS followed by HPLC with fluorescence and UV-diode array detector (DAD). Approximately 500 g of each sample was comminuted and 10 g of each was transferred into 50 ml polyethylene tube, followed by addition of 15 ml of acetonitrile and 5 ml of deionized water. The mixture was shaken using a vortex and 6 g of anhydrous magnesium sulphate and 1.5 g of sodium chloride were added and shaken vigorously for 5 min, then centrifuged at 4000 rpm for 4 min. A 4 ml aliquot of the supernatant was transferred to 15 ml centrifuge tube containing 100 mg of PSA and 600 mg of anhydrous magnesium sulphate. The mixture was again vortexed for 3 min and centrifuged for 10 min at 4000 rpm. The supernatant containing the target analytes was derivatized by addition of 50 μl of TFA and 200 μl of hexane, vortexed for 5 min and 1.95 ml of acetonitrile/water (1:9) was added and then centrifuged for 3 min at 4000 rpm. The supernatant was then subjected to HPLC analysis with C18 column and water/methanol/acetonitrile (65/23/12) used a mobile phase at flow rate of 1 ml/min. The recovery results of total aflatoxins and carbamate pesticides were found to range from 88.25 to 92.9% and 78.49 to 98.11%, respectively. The results indicated that all samples were free from any detectable aflatoxin (B1, B2, G1 and G2). On the other hand, promocarb, pirimicarb and aldicarb residues were found in few bee honey samples. All contaminated bee honey samples with carbamate pesticides were under maximum residue limit (MRLs) [26].

A reliable and easy method was developed for the determination of aflatoxins B1 and G1 in maize samples. The mycotoxins content of maize was extracted using QuEChERS coupled to HPLC-FLD with photochemical derivatization. The method used involved weighing 10 g of maize sample into a centrifuge tube and shaking vigorously for 1 min, followed by addition of 1.67 g of sodium acetate and then shaking again for 2 min, the 10 ml 1% acetic in MeCN and 5 ml H_2O of water were added. About 4 g of $MgSO_4$ was added and the mixture was centrifuged for 20 min at 3000*g*. The upper layer was then submitted for HPLC analysis without the clean-up step. The method validation gave linearity between 0.4–20 μg/kg with correlation coefficient greater than 0.99. The limit of detection and quantification were estimated to be 0.08–0.16 and 0.4 μg/kg, respectively, while the average recovery ranged from 79.5–99.73% with RSD ranging from 1.10 to 2.27%. It was discovered that when acetic acid was used with acetonitrile for partitioning, further clean-up is not required, which saves analysis time [27].

Aflatoxins M1, M2, B1, B2, G1, G2 and ochratoxin A were determined in UHT and powdered milk using the modified QuEChERS method coupled to ultra-high performance liquid chromatography–tandem mass spectrometry. For powdered milk, 1.5 g of the sample was transferred into a 50 ml centrifuge tube, followed by addition of 15 ml of deionized water, and the tube was shaken for 30 s. Then, 10 ml of hexane and 15 ml of acetonitrile containing 1% acetic acid were added, followed by 6 g of magnesium sulphate and 1.5 g of sodium chloride and the tube was vigorously shaken for 1 min and then centrifuged for 7 min at 3000 rpm. After centrifugation, the upper layer of hexane was removed and an aliquot of 5 ml of acetonitrile layer was concentrated to dryness with an evaporator at 50°C under gentle flow of nitrogen. The residue was dissolved with 1 ml of mixture of methanol and water (1:1), and the solution was filtered through 0.22 μm polyethylene filter. The filtrate was then subjected to UHPLC procedure using a solution of 5 mM of ammonium formate and 1% acetic acid (phase A) and methanol (phase B) as the mobile phase at a flow rate of 0.3 ml/min. The method yielded good linearity which ranged from 0.1 to 1.5 ng/ml

with r^2 greater than 0.99. The LOQ ranged from 0.005 to 0.44 μg/kg, while the LOD ranged from 0.017 to 1.45 μg/kg. The average recoveries for the two types of milk sample range from 72.8 to 121% with RSD = 0.7–16.7%. The analysis of real sample showed the absence of ochratoxin A, aflatoxins B1, B2, G2 and G2 in the milk samples, while aflatoxins M1 were found at concentration levels ranging from 0.005 to 0.0043 and 0.08 to 1.19 μg/kg in UHT and powdered milk, respectively. The aflatoxins found in the milk sample were below the maximum permitted level according to Brazilian legislation, but high according to the EC regulation [28].

The application of QuEChERS sample preparation was optimized and validated for the analysis of mycotoxins in brown rice. The brown rice was blended to a powder sieved and homogenized. An aliquot of 1 g of the homogenized powder sample was transferred into a 50 ml centrifuge tube and 5 ml of water was added and mixed. A 5 ml solution of acetonitrile containing 10% acetic acid was then added to the mixture and vortexed for 1 min at high speed. After the vortexing, 2.0 g anhydrous $MgSO_4$, 0.50 g NaCl, 0.50 g sodium citrate tribasic dihydrate and 0.25 g sodium citrate dibasic sesquihydrate were added and the mixture was vigorously shaken for 1 min, and then centrifuged for 5 min at 1911 × *g*. The supernatant (2 ml) was then transferred into a 15 ml centrifuge tube containing 300 mg anhydrous $MgSO_4$, 50 mg C18, 25 mg of PSA and 25 mg silica. This portion was shaken and centrifuged, and then 1 ml of the supernatant was evaporated to dryness under a stream of nitrogen gas. An aliquot was reconstituted in 1 ml of water with a 1:1 (v/v) ratio of 0.1% (v/v) FA:MeOH and 0.5 μg/l of an SMX IS. The extracted solutions were filtered through 0.22-μm PTFE syringe filters prior to UHPLC–MS–MS analysis. The analytical limits obtained from the method using internal standard calibration method gave linearity in the range of 5–1000 μg/kg, with limit of detection and limit of quantitation ranging from 1.4 to 25 μg/kg and 4.1–55 μg/l, respectively and recoveries in the range of 81–101% with relative standard deviations of 5–19%. Six out of 14 real samples of brown rice were found to be contaminated with at least one of these mycotoxins, ranging from 2.49–5.41 μg/kg of FB1, 4.33 ± 0.04 μg/kg of FB2 and 6.10–14.88 μg/kg of ZON [29].

Aflatoxins were determined in wheat and wheat by-products using in-house validation methods. Three different methods were compared in the study; method 1 involved extraction with chloroform and removal of interfering chemicals by filtration, liquid–liquid partition with hexanemethanol–water and methanol–water-chloroform, and pre-column derivatization with trifluoroacetic acid [30], Method 2 involved extraction with methanol and KCl, purification by filtration with $(NH)_4SO_4$ and Celite, liquid–liquid partition with methanol–water-hexane and methanol–water-chloroform with precolumn derivatization with trifluoroacetic acid; and method 3 involved extraction with methanol:water:acetonitrile (51:40:9, v/v/v) $MgSO_4$ and NaCl, followed by centrifugation and filtration, and the quantification was carried out by HPLC-FLD, without derivatization [31]. Method 3 involved weighing of 2.0 g of thoroughly homogenized sample into a 15 ml polypropylene centrifuge tube followed by addition of 10 ml of extraction solution containing a mixture of methanol:water: acetonitrile (51:40:9, v/v/v) and manually stirred for 1 min. Subsequently, 1.5 g of anhydrous $MgSO_4$ and 0.5 g of NaCl were added and then shaken manually for 1 min. Afterwards, the tube was centrifuged for 5 min, at 4000 rpm, and 1 ml of the extract was collected, filtered through a 0.45 μm membrane and injected into the HPLC-FLD system, without any derivatization procedure. The quantification of the aflatoxins was carried out in an HPLC system, using a fluorescence detector with C18 column isocratic mobile phase, consisting of water: methanol: acetonitrile, at a flow rate of 1.0 ml/min. The methods were validated accruing to the European Commission

method EC/401/2006 [32]. The average recoveries were found to be highest in method 1, followed by method 2 and the least was found in method 3, which was observed to be due to lack of derivatization. The method showed a relative standard deviation (RSD) lower than 15% and recovery values in the 70−110% range, with linearity between 1.2 and 24 μg/kg, while the limits of detection and quantification (0.6 and 1.2 μg/kg, respectively) were below the maximum level of aflatoxins allowed in wheat and wheat by-products by the European Commission (4.0 μg/kg) and by the Brazilian legislation (5.0 μg/kg). Using the validated method, aflatoxins were quantified in 20 commercial samples of wheat grains, wheat bran, whole wheat flour and refined wheat flour intended for direct human consumption. Six samples (30%) were positive for aflatoxins and all samples presented levels below the maximum limit stipulated by the Brazilian legislation [33].

QuEChERS LC–MS/MS method was applied for the screening of 12 mycotoxins in cereal products and spices. The samples were homogenized at ambient temperature and 5 g of homogenized samples were separately weighed into a falcon tube and fortified with the working standard solution and left for 10 min. After 10 min, 10 ml of double distilled water and 10 ml of acetonitrile containing 20% acetic acid were added. The mixture was vortexed for 15 min and left for 15 min at −20°C. This was followed by addition of 4 g of $MgSO_4$, 1 g of NaCl and 1 g of sodium citrate tribasic dehydrate and the mixture was shaken for another 1 min and then centrifuged for 10 min at 5000 rpm. The supernatant was transferred into a tube containing 900 mg $MgSO_4$, and 150 mg Supelclean PSA. The mixture was hand shaken and subsequently centrifuged for 5 min at 5000 rpm. An aliquot of 3 ml of the supernatant was evaporated and redissolved in 600 μl of methanol/water (50/50 v/v). The final solution was subjected to HPLC analysis with mobile phase consisting of water (A) and methanol (B). The average recovery of the developed method ranged from 60 to 120% with RSD between 0.026 and 36.7%. The method was found to satisfy the requirements of Commission Regulation (EC) No. 401/2006 and (EC) no. 1881/2006. The screening target concentration (STC) was under maximum permitted levels (MLs) for all mycotoxins validated. All samples were compliant and followed (EC) no. 1881/2006. One sample of maize resulted in OTA at 2.53 μg/Kg, and one sample of black pepper resulted in 1.85 μg/Kg of OTA and the contemporary presence of 0.358 μg/Kg of AFB2 [34].

A reliable and rapid method has been developed for the determination of aflatoxin B1 (AFB1) in four kinds of feedstuffs comprising broken rice, peanuts, corn, and fishmeal. Sample preparation was carried out based on the QuEChERS method with the exclusion of the clean-up step. In this study, AFB1 was extracted using acetonitrile/methanol (40/60 v/v), followed by partitioning with sodium chloride and magnesium sulfate by measuring 10 g of well-milled and homogenized sample into extraction tube followed by addition of 20 ml of acetonitrile/methanol (40/60, v/v%) and the mixture was centrifuged for 3 min at 3000 rpm. Thereafter, 1 g of NaCl and 4 g of anhydrous magnesium sulphate were added and the mixture was shaken and centrifuged again at 3000 rpm for 3 min. The supernatant (1 ml) was evaporated until dry under nitrogen gas. Following that, the precolumn derivatization of AFB1 was carried out and the residue was reconstituted in 900 μl of 10% acetonitrile followed by addition of 100 μl of trifluoroacetic acid and then incubated for 15 min at 15°C. The derivatized solution was then centrifuged at 1000*g* for 5 min before HPLC-FLD analysis. The method validated yielded recovery of all feedstuffs achieved a range of 82.50–109.85% with relative standard deviation ranging from 0.57−11% for all analytes at a concentration of 20−100 ng/g. The limit of detection (LOD) ranged from 0.2 to 1.2 ng/g and limit of quantitation (LOQ) ranged from 0.3 to 1.5 ng/g. The

validated method was successfully applied to a total of 120 samples. The occurrence of AFB1 contamination was found at the following concentrations: in broken rice (0.44−2.33 ng/g), peanut (3.97−106.26 ng/g), corn (0.88−50.29 ng/g), and fishmeal (1.06−10.35 ng/g). It was suggested as an alternative to expensive and time-consuming methods by using immune affinity columns or two steps of liquid/solid extraction procedure [35].

A rapid method is proposed for determining aflatoxins B1 and M1 in milk and dairy products by HPLC with fluorimetric detection. A sample of about 5.0 g was collected into a 50 ml test tube and 5 ml of water and 10 ml of acetonitrile were added; the tube was sealed and shaken for 30 min. Then a mixture of salts consisting of 1.0 g of NaCl, 1.0 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate was added. The test tube was shaken for 1 min and centrifuged for 45 min at 5000 rpm. An 8.0 ml portion of an extract from the upper layer was collected and taken into a 15 ml centrifuge tube, already charged with a mixture of 950 mg of $MgSO_4$, 200 mg of adsorbent Bondesil PSA, and adsorbent C18 (200 mg, for raw milk or dairy products or 400 mg for cheese). The tube was shaken vigorously for 30 s and centrifuged for 5 min at 2700 rpm. Then 3.0 ml of an acetonitrile extract and 500 μl of chloroform were put into a 15 ml centrifuge tube charged with 7.0 ml of deionized water using a syringe. The mixture was shaken for 20−30 s, kept in an ultrasonic bath for 2 min, and centrifuged for 10 min at 2700 rpm; the bottom layer was collected into a microvial and evaporated to dryness in a flow of nitrogen; the residue was dissolved in 50 μl of acetonitrile and subjected to chromatography analysis. The method gave average recoveries ranging from 51.2–75.7%. The limit of detection and quantitation were estimated to range from 0.01−0.1 and 0.03–0.3 μg/kg, with linearity ranging from 0.03 to 10 μg/kg and a correlation coefficient greater than 0.998. Aflatoxin B1 was found in samples of cheese only, and M1 was found in all the samples studied. The concentration of aflatoxins did not exceed the maximum permissible concentration legalized in Russia [36].

A suitable method for routine analysis of aflatoxins M1, M2, B1, B2, G1 and G2 in peanut by ultra-high performance liquid chromatography–tandem mass spectrometry was developed and validated. The sample preparation was performed using a triple partitioning (water/acetonitrile/hexane) modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method. For the first time, this method is reportedly used for aflatoxins analysis in peanuts. To 5 g of the sample, weighed in a 50 ml centrifuge tube, were added 10 ml of ultrapure water, 10 ml of hexane and 15 ml of acetonitrile; the tube was then shaken for 30 s; a mixture of 4 g of magnesium sulphate and 1.5 g of sodium chloride was added, the tube was immediately shaken vigorously using a vortex for1 min and then centrifuged at 3000 rpm for 7 min. An aliquot of 5 ml of the acetonitrile phase was evaporated to dryness under a gentle flow of nitrogen at 45°C and then the residue was dissolved with 2 ml of methanol/water (1:1, v/v). The solution thus obtained was filtered through a 0.22 μm polyethylene filter before injection to HPLC. Satisfactory recoveries ranged from 71.3 to 101.3%, with a relative standard deviation ranging from 1.5–12.4% obtained for the target aflatoxins. The determination coefficients were ≥ 0.99 which showed good linearity (0.15–15 μg/l). The LOD and LOQ varied from 0.03 to 0.26 ng/g and 0.1 to 0.88 ng/g, respectively [37].

Two multi-residue methods were developed and compared for the analysis of 17 mycotoxins in cereals by liquid chromatography-electrospray ionization tandem mass spectrometry. The extraction procedures considered were a QuEChERS-like method and one using accelerated solvent extraction (ASE). The QuEChERS-like extraction procedure involved weighing 5 g of the sample into a 50 ml tube followed by addition

of 10 ml of water and 10 ml of acetonitrile containing 0.5% acetic acid. The mixture was vigorously shaken and 5 g of $MgSo_4$/NaCl (4:1, w/w) was added and shaken again, followed by centrifugation at 4000 rpm for 15 min. For clean-up, 5 ml of the supernatant was transferred into a 15 ml tube and was defatted with 5 ml of n-hexane under agitation and then centrifuged for 1 min at 4000 rpm. Subsequently, 1 ml of the supernatant (equivalent to 0.5 g of sample) was transferred into a tube and evaporated to dryness at 40°C under stream of nitrogen and the residue was reconstituted with 75 µl of methanol, sonicated for a few min and 75 µl of water was added. The whole extract was then transferred into a 1.5 ml tube and centrifuged at 8500 rpm for 10 min. The resulting supernatant (60 µl) was then further diluted with water (140 µl) and recentrifuged (8500 rpm 10 min), and the clear supernatant was transferred into an HPLC amber glass vial for further LC-ESI-MS/MS analysis. The method validation estimated using the optimized method gave recovery ranging from 73 to 130% with RSD of 0 to 18%. The LOQ was between 0.5–100 µg/kg. The two-extraction procedure was found to give similar performances in terms of linearity ($r^2 > 0.98$), both methods showed high extraction efficiency in a broad range of cereal-based products and with comparable sensitivity. Nevertheless, the easiness-to-handle of these extraction methods was definitely in favor of the QuEChERS-like procedure, since it requires less reagents and glassware and involves less intermediate steps. Consequently, a higher sample throughput was possible, with up to 40 individual samples extracted over one working day as compared to the 24 individual samples processed over one and a half working days by the ASE procedure. On a routine basis, the QuEChERS-like method constitutes undeniably the best option [38].

The presence of mycotoxin and pesticide residues was analyzed in milk using QuEChERS method. The efficiency was evaluated using the original QuEChERS and acetate buffered methods. For the original method, 10 ml of milk was extracted with 10 ml of acetonitrile, stirred for 1 min, followed by addition of 1 g of NaCl and 4 g of $MgSO_4$, with stirring at vortex for 1 min and centrifuged at 5000 rpm for 5 min. And for the acetate buffered QuEChERS method, 15 ml of milk was extracted with 15 ml of acetonitrile containing 1% acetic acid, the mixture was stirred for 1 min, followed by addition of 6 g of $MgSO_4$ and 1.5 g of sodium acetate with stirring at vortex for 1 min and centrifuged at 5000 rpm for 5 min. After centrifugation, 2 ml of the supernatant was transferred to a centrifuge tube containing 150 mg of $MgSO_4$, 50 mg of PSA and 50 mg of C18. The mixture was stirred for 30 s and centrifuged at 5000 rpm for 5 min. The extract was filtered through a PTFE membrane, and then 1 ml of extract was transferred to a vial, evaporated to dryness and redissolved in 1 ml of mixture of acetonitrile/ammonium formate +0.01 formic acid (95/5 v/v) and subjected to UPLC. The original QuEChERS method was found to be more efficient than the acetate buffered and was adopted for method validation. The developed method was validated according to the analytical quality assurance manual of the Brazilian Ministry of Agriculture and the European Commission Decision No 2002/657/EC. The average recovery values were found between 85 and 97% for aflatoxin M1, with RSD ranging from 14.5 to 16.3%. The limit of detection and quantification were 0.02 and 0.04, with linearity ranging from 0 to 1.0 µg/kg and correlation coefficient of 0.997. Residues of aflatoxin M1 were also found in field samples at levels below the established maximum residue limit [39].

A simultaneous analysis method was developed for faster and cheaper determination of 13 different mycotoxins in feedstuffs using QuEChERS followed by LC–MS/MS. For sample preparation, 5 g of the freeze-dried samples were accurately weighed and transferred into 50 ml tube followed by addition of 10 ml of water containing 10% formic acid, and 10 ml of acetonitrile. The mixture was shaken for 30 min, and

different combinations of salts were then added (salt 1 containing 4 g anhydrous magnesium sulfate and 1 g sodium chloride; salt 2, 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate; salt 3, 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate). The mixture was shaken for 1 min and then centrifuged at 4000 rpm for 10 min. For the cleanup, 1 ml of the supernatant was transferred into a tube containing 25 mg of PSA and 25 mg of C18 and then centrifuged for 5 min at 10,000 rpm. A 400 μl aliquot of the supernatant was then transferred to a microtube and mixed with 500 μl of distilled water and 100 μl of acetonitrile. The solution was then filtered through 0.20 μl PTFE syringe filter and was then subjected to LC–MS/MS analysis. The salt containing magnesium sulfate and sodium chloride (salt 1) was found to be the most efficient and was used for method validation. The analytical method was validated following SANTE/11813/2017 and CODEX guidelines. Average recovery was found between 70.1 and 115.6% with RSD –0.1−11.3, the LOD ranged from 0.8333 to 16.7 μg/l, the LOQ ranged from 2.5 to 50 μg/l, while linearity ranged from 0.5 to 500 μg/l with r^2 greater than 0.99. Mycotoxins were found in the 39 samples but did not exceed the maximum residual level (MRL) criterion set by Korean Food and Drug Administration [40].

A simple and efficient method for determining multiple mycotoxins was developed using a QuEChERS-based procedure for the analysis of mycotoxins in vegetable oil using high-performance liquid chromatography–tandem mass spectrometry. Different extraction procedures were studied and optimized by spiking 16 analytes into blank matrix. A 1 g sample was weighed into a 30 ml centrifuge tube and then spiked with a mycotoxin's standard mixture at different concentrations and was left for 1 h for equilibration. Then 2 ml of water was added vortexed for 1 min and 8 ml of acetonitrile was thereafter added and the extraction was achieved using end-over-end shaker for 20 min. Subsequently, 4 g of anhydrous Na_2SO_4 and 1 g of NaCl were added. The tube was capped immediately, vortexed for 2 min and then centrifuged at 5000 rpm for 5 min. The supernatant (8 ml) was transferred into a 15 ml centrifuge tube containing different sorbents (C18, PSA and neutral Al_2O_3, containing 100, 150 and 200 mg, respectively) and the tube was shaken by hand for 5 min and centrifuged for 5 min at 8000 rpm and 4 ml of the extract (upper layer) was transferred into a glass tube and evaporated to dryness under a stream of N_2 and then reconstituted by addition of 1 ml of mixture of acetonitrile/water (1:1, v/v%). The most efficient extraction was achieved with 85% of acetonitrile solution and C18 as cleanup sorbent, which allowed average recovery between 72.8–105.8% with RSD less than 7% (RSD = 0.2−6.3). The limit of detection (LOD) ranged from 0.04 to 2.9 ng/g, while limit of quantitation ranged from 0.12 to 10 ng/g, with linearity ranging from 0.2 to 500 ng/g and r^2 greater than 0.99. Zearalenone (ZEN), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1(AFG1) and α-zearalenol (α-ZOL) were detected, with maximum concentrations of 0.59 (AFG1)– 42.5 (ZEN) ng/g. The method developed has the advantages of high sensitivity, accuracy and selectivity, and it can be applied to the target screening of mycotoxins in real samples [41].

A new method for the determination and analysis of ochratoxin A (OTA) in cereals and cereal products, based on the use of the QuEChERS procedure enhanced with HPLC-FLD was developed. Cereal samples were prepared similar to the previous published aflatoxin QuEChERS method with some modifications. The modified QuEChERS involves three steps; First step includes measuring a thoroughly homogenized cereal sample (1 g) weighed in a polypropylene centrifuge tube (15 ml). Subsequently, they were extracted via the following steps (II to IV). Step II**:** 3.0 ml of

20:70:10 (%, v/v) water/acetonitrile/acetic acid mixture was added, and the centrifuge tube was shaken for 1 min to ensure that the solvent has mixed thoroughly with the entire sample, for complete extraction of the analyte. Step III: 0.8 g of anhydrous MgSO4 and 0.2 g of NaCl were added to the mixture and the shaking procedure was repeated for 1 min to facilitate the extraction and partitioning of the ochratoxin A into the organic layer. Step IV: The extract was centrifuged for 5 min at 4000 rpm and 0.5 ml of the upper organic layer was filtered through a 0.45 μm nylon syringe filter prior to HPLC analysis. The linearity of the developed method ranged from 3.75 to 120 μg/l with r^2 greater than 0.99. The recoveries obtained ranged from 85.2 ± 1.2 to 109.8 ± 2.9%, with a relative standard deviation (RSD) of less than 12%. The LOQ were from 0.60 to 2.08 μg/kg, with LOD ranging from 0.18 to 0.62 μg/kg [42].

A combination of modified QuEChERS with ultrahigh-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) has been used for simultaneous detection of 20 mycotoxins in grains. A series of different types of magnetic (Fe_3O_4) nanoparticles modified with multiwalled carbon nanotubes (Fe_3O_4-MWCNTs) were designed as modified QuEChERS adsorbents for facile and efficient purification and for target interferences removal in the matrices. A 5.0 g of grains was added into 50 ml centrifugation tubes. Acetonitrile/water (25 ml; 80:20, v/v, 1% acetic acid) was added, and the tube was vigorously shaken with a vortex mixer for 1 min. Then, after citrate buffer containing 4 g of MgSO4, 1 g of NaCl, 1 g of $Na_3Cit{\cdot}2H_2O$, and 0.5 g of $Na_2Cit{\cdot}1.5H_2O$ were added, the mixture was shaken vigorously for 1 min followed by centrifugation at 8000 rpm for 5 min. Fe_3O_4-MWCNTs (20 mg) and 1.0 ml of the purified supernatant were added to a 2.0 ml microcentrifuge. The mixture was mixed vigorously for 1 min and separated by a magnetic force created by a magnet. The solution was passed through a 0.22 μm PTFE membrane filter, and 5 μl of the final solution was analyzed by UPLC -MS/MS. The method validated in accordance with the Commission regulation 401/2006/EC and SANCO guidelines 12,571/2013 gave linear range of 0.1–500 ng/g with r^2 greater than 0.99. The method yielded good recovery between 73.5 and 112.9% and RSD ranging from 1.3 to 12.7%, with the LODs and LOQs for the 20 mycotoxins ranging from 0.0006 to 1.6337 and from 0.0021 to 5.4457 ng/g, respectively. The developed method was compared with published works, where other adsorbent materials and the developed method were found to have a wider linear range and lower LOQ [43].

Five mycotoxins were detected in different food matrices obtained from Malaysian market using validated QuEChERS-LC–MS/MS. Low-fat samples were prepared by measuring 2 g of homogeneous solid food (or 2 ml liquid sample) were weighed and transferred to a 50 ml centrifuge tube. Then, 10 ml of acetonitrile acidified with 1% acetic acid and 7.5 ml of cold water were added to the tube, shaken for 1 min, and vortexed for 4 min, followed by the addition of 4 g of anhydrous $MgSO_4$ and 1 g of sodium chloride and shaken for 3 min. The mixture was then centrifuged for 6 min at 7500 rpm. Exactly 4 ml of the supernatant was pipetted out and added to a 15 ml centrifuge tube containing 0.2 g PSA and 0.6*g* of fine powder anhydrous $MgSO_4$. The extract was further shaken for 2 min and centrifuged at 4000 rpm for 5 min. Then, 2.5 ml of the supernatant was evaporated to dryness by a rotary evaporator and reconstituted with 1 ml of methanol and filtered through a 0.22 μm nylon syringe filter prior to the LC–MS/MS analysis. For samples with high-fat content, 2.5 g of the homogenized samples was weighed and transferred to a 50 ml polypropylene centrifuge tube. Then, 20 ml aqueous acetonitrile (containing 1% acetic acid) solution (80:20, v/v) was added to the mixture and shaken for 30 min at 300 rpm. The mixture was then centrifuged for 5 min at 8000 rpm and the supernatant was transferred into a clean vial.

The extraction process was repeated twice. Then, 4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate, and 0.5 g of sodium hydrogen citrate sesquihydrate were added to the combined supernatant and shaken for 1 min. The fat content was removed by treating the extracts with 20 ml of hexane (2 times), vortexing for 1 min and followed by standing for 5 min to separate the hexane from the extract. For the dispersive SPE clean-up, the bottom layer was transferred into a clean tube that contained 150 mg of C18 sorbent and 900 mg of magnesium sulfate. The cloudy solution was shaken for 1 min and centrifuged at 8000 rpm for 5 min. The supernatant was transferred into a clean tube and washed twice with 5 ml of acetonitrile. The mixture was evaporated to dryness by a rotary evaporator and reconstituted with 1 ml of methanol and filtered through a 0.22 μm nylon syringe filter prior to LC–MS/MS analysis. The method demonstrated good sensitivity of concentration range ranging from 1 to 30 μg/kg ($r^2 > 0.996$), with LOD that ranged from 0.05 to 0.1 μg/kg, and LOQ that ranged from 0.08 to 0.3 μg/kg, which was found to be lower than the allowable maximum limit for aflatoxin. The recovery of the target analytes ranges from 81.94 to 101.67%, intra-day and inter-day precision range from 0.12 to 7.25% and 0.23 to 10.28%, respectively. The developed method was compared to other QuEChERS methods, and the developed method revealed excellent overall results. Aflatoxins were detected in raisin, pistachio, peanut, wheat flour, spice, and chili samples with concentrations ranging from 0.45 to 16.93 μg/kg. Trace concentration of ochratoxin A was found in wheat flour and peanut samples which ranged from 1.2 to 3.53 μg/kg. Some of the tested food samples contained mycotoxins above the European legal maximum limit [44].

A method was developed to simultaneously quantify different mycotoxins in 30 and 10 corn flour samples using modified QuEChERS in combination with an LC–MS/MS technique. About 5 g of homogenized corn flour sample was placed into the falcon tube (50 ml). The appropriate concentrations of the mixed working standard solution (for spiking) and aflatoxin M1 (internal standard) were added to the falcon tube. After an hour, 15 ml of acetonitrile (79%):water (20%):formic acid (1%) (v/v/v) were added, this was followed by addition of 1 g of sodium chloride. The obtained mixture was shaken vigorously for 10 min at 340 rpm. Then, 5 g of magnesium sulfate was added to the mixture. Subsequently, the sample was vortexed for 2 min and centrifuged for 10 min at 1585*g* at 5°C. An aliquot of 5 ml of the extract was transferred to a Falcon (15 ml) tube containing 1 g $MgSO_4$ and 0.3 g PSA Again, the samples were vortexed and centrifuged as described previously. Afterwards, 4 ml of the extract was poured into a vial and then evaporated to dryness using nitrogen gas. Afterwards, reconstitution of the residue using methanol (1 ml) was carried out. Then, 20 μl of solution was injected into the LC–MS/MS after filtering through 0.2 μm syringe filter. The method validated using SANTE/11945/2015 document gave linearity between 2 and 1800 ng/g with r^2 greater than 0.99. The LOQ and LOD were respectively found 2–75 ng/g and 0.6−25 ng/g. The recoveries were in the range 92.9–103.8% and RSD 3.7–20%. AFB1, OTA, and ZEA were detected and quantified in 23 (76.6%), 6 (20%), and 14 (46%) of 30 samples, with average contamination of 154.1, 25, and 358.7 ng/g, respectively. The co-occurrence of AFB1 + ZEA and AFB1 + OTA + ZEA was noted in 20% and 23% of corn samples, respectively. The measured level of contamination for DON and T-2 toxin in corn flour samples did not exceed the maximum tolerated level [45].

A fast, easy, and cheap method for the simultaneous determination and quantification of aflatoxins in cereal-derived products was developed by Annumziata and co-workers using QuEChERS extraction coupled with LC–MS/MS. The sample was prepared by measuring 2 g of the homogenized sample into a 50 ml tube and was spied with the internal standard. The fortified sample was kept in the dark for 15 min, to

allow equilibration of IS with the matrix. Then 10 ml of water containing 0.1% formic acid was added and shaked the mixture for 3 min. Then 10 ml of acetonitrile were added, and the sample was further shaken for 3 min. This was followed by addition of 4 g MgSO4 and 1 g NaCl and the mixture was immediately shaken for 2 min to prevent agglomerates from forming during MgSO4 hydration and then centrifuged at 3500 × *g* for 10 min. About 2 ml of the extract was evaporated under a stream of nitrogen and the residue was constituted by addition of 200 μl of a solution of methanol/water containing 5 mM ammonium formate and 0.1% formic acid (10:90, v/v) and was then filtered through a 0.45-μm polyvinylidene fluoride filter into a vial. The developed method was validated using regulation EC 888/2004, and it yield average recoveries ranging from 83.8 to 102.9% with RSD between 14.3 and 15.7%. The LOD and LOQ were 0.5–100 and 1–200 μg/kg, respectively, and linearity ranging from 0 to 140 μg/kg with r^2 greater than 0.99. The method was then applied for the analysis of 21 cereal-derived products purchased on the Italian market, which were correctly packaged and labeled as intended for human consumption. The co-occurrence of more than one mycotoxin in the analyzed samples could represent a risk for consumers, and the described method could be a valid alternative for their simultaneous detection in the framework of official control [46].

The possibility of applying QuEChERS extraction of 2 mycotoxins in cereals and subsequent detection using enzyme-linked immunosorbent assay (ELISA) has been investigated. Each homogenized cereal sample was accurately weighed (4.0 g, precision: 0.01 mg) into 50 ml centrifuge tubes, followed by addition of 16 ml of mixture of acetonitrile/methanol (80:20 v/v) and the mixture was shaken for by vortex for 1 min. Then QuEChERS extraction kit (6 g of magnesium sulphate, 1.5 g of sodium chloride, 1.5 g of sodium citrate dihydrate and 1 g of sodium citrate sesquihydrate) was added and the tube was shaken vigorously for 5 min and then centrifuged at 8000 rpm for 10 min. An aliquot of 8 ml of the supernatant was transferred into 15 ml centrifuge tube containing 300 mg PSA and 100 mg $MgSO_4$ anhydrous and the tube was vortexed vigorously for 1 min. Subsequently, the sample was centrifuged at 8000 rpm for 3 min. And then, 4 ml of the upper organic solvent layer was transferred to a vial, evaporated to near dryness under a gentle stream of N_2 and reconstituted with 1 ml of MeOH: PBS (10:90, v/v) for ELISA analysis. The recovery of the method ranged from 83.55 to 106.93% with RSD between 1.11 and 7.42%. The sensitive and specific ELISA was applied to the determination of ZEN and DON in cereal. For ZEN, the linear range was 13.64–104.48 ng/ml, the LOD was 2.58 ng/ml, and for DON, the linear range was 35.65–983.52 ng/ml, the LOD was 17.31 ng/ml. The ELISA method for determination of ZEN and DON was compared with a standard HPLC method. The values obtained from the two detection systems for ZEN and DON entirely fit an excellent linear relationship, with a regression equation of $y = 1.0281x - 0.2897$ (correlation coefficient is 0.9955) for ZEN and a regression equation of $y = 0.9952x + 4.4193$ (correlation coefficient is 0.9984) for DON, further confirming the reliability of ELISA [47].

A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was developed for the simultaneous determination of veterinary drugs, pesticides and mycotoxins in eggs by ultrahigh-pressure liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). In the purification procedure, magnetic multiwalled carbon nanotubes (Fe_3O_4-MWCNTs) were used as adsorbents, and an external magnet was utilized to achieve a faster adsorbent separation, compared to the traditional centrifugation process. About 5.0 g of the homogenized sample were weighed into a 50 ml centrifuge tube. Subsequently, 5 ml of 0.1 mM Na_2EDTA solution and 20 ml of 1% of HOAc in ACN were added. The mixture was then vortexed for 1 min, followed by

addition of 4.0 g of Na_2SO_4 and 1.0 g of NaCl, and the tubes were vortexed immediately for another 1 min. The sample was then centrifuged at 6000 rpm for 10 min, and 2 ml of supernatant was transferred into a 5 ml dispersive tube containing 15 mg of Fe_3O_4-MWCNTs composite. The mixture was vortexed for 1 min, and then the adsorbents were separated quickly from the solution with an external magnet. Finally, 1 ml of the above solution was diluted with 1 ml of aqueous methanol solution (50/50, v/v), and the solution was finally filtered by a 0.22 μm PTFE syringe filter for UPLC–MS/MS analysis. The recoveries of all analytes were in the range of 60.5−114.6%, while the recoveries for the mycotoxins varied from 71.8 to 100.0%, at three fortified levels with relative standard deviations (RSDs) ranging from 1.6 to 17.3%. The LOQs for all the target analytes ranged from 0.1 to 17.3 μg/kg, while it was found between 0.2 and 11.8 for mycotoxins and the linearity ranged from 1 to 100 μg/kg. This method was successfully applied to the analysis of egg samples, demonstrating its applicability and suitability for the routine analysis of multiclass residues in egg samples [48].

A high-throughput method for the simultaneous determination of 26 mycotoxins in sesame butter was developed by coupling the modified QuEChERS method with ultra-high performance liquid chromatography triple quadrupole mass spectrometry (UHPLC–MS/MS). The sample (2.5 g) was weighed into a centrifuge tube and sequentially extracted using two different solutions. The sample was first extracted using 20 ml of a mixture of acetonitrile/water solution (80:20, v/v) containing 0.1% formic acid for 30 min by continuous shaking. The tube was shaken with shaker at 300 rpm, followed by centrifugation at 8000 rpm for 5 min. The supernatant was then transferred to a clean vial. The remaining residue was further extracted using 5 ml of an acetonitrile aqueous solution (20:80, v/v) containing 0.1% formic acid for an additional 30 min with continuous shaking at 300 rpm, followed by centrifugation at 8000 rpm for 5 min. The two supernatants were combined before being subjected to salting out and fat removal. About 4 g of magnesium sulfate, 1 g of sodium chloride, 1 g of sodium citrate and 0.5 g of sodium hydrogen citrate sesquihydrate was added to the supernatant with immediate vortexing for 1 min to enhance the partition of the mycotoxins into the organic layer. The tube was then centrifuged at 8000 rpm for 5 min. The upper layer was collected and mixed with 20 ml of hexane, followed by vortexing for 1 min to remove fat. After standing for 5 min, the upper layer was removed, and the lower layer was transferred into a tube containing 150 mg of C18 and 900 mg of magnesium sulfate for the dSPE clean-up. The cloudy solution was vortexed for 1 min and then centrifuged. The resulting supernatant was decanted into a clean tube. The dispersive tube was washed twice with 5 ml of acetonitrile, sequentially. The washing solution was then collected and combined with the supernatant. The resulting sample solution was subjected to drying at 40°C using the rotary evaporator. Finally, the residue was sequentially dissolved in 1.5 ml of methanol and 1.0 ml of water, and the resultant solution was passed through a 0.22-m nylon filter for further analysis using UHPLC–MS/MS. The calibration curves were prepared in a blank matrix with a series of concentrations between 0.5 and 500 ng/ml with good linear relationships were achieved with linear regression coefficients (r^2) of 0.994 or higher. The LOQs of the samples ranged from 0.11 to 21.74 μg/kg, while the LOD ranged from 0.05 to 7.25 μg/kg. The recovery values (60–111.70%) are within 60–120%, except those of FB2 and PAX at two higher levels and that of ST at the lowest level. All of the RSDs (0−14.6%) were within 15%, with the majority of values (96% of the total) within 10%. The results showed that 10 peanut butter samples were all contaminated with AFs and FBs with total concentrations in the range of 2.4–4.6 and 6.9–20.1 μg/kg, respectively. Other mycotoxins were not detectable in peanut butter [49].

A fast analytical method for the simultaneous determination of 9 mycotoxins in corn using dSPE and ultra-performance liquid chromatography coupled to tandem quadruple time-of-flight mass spectrometry (UPLC-Q-TOFMS) was developed and validated by Wang et al. (2016). The corn samples (2.00 g ± 0.01 g) were accurately weighed in a 50-ml polypropylene tube. The samples were extracted with 20 ml of acetonitrile-water (84:16, v:v) containing 1% acetic acid, in an ultrasonic water bath for 20 min at room temperature. $MgSO_4$ (2 g) and NaCl (0.5 g) were added, and the tube was shaken vigorously for 1 min and then centrifuged 5000 rpm for 5 min. The supernatant was transferred to a separate centrifuge tube each containing C18 powder, PSA and GCB, (with 30 mg of C18 giving highest recovery) and shaken for 1 min and then centrifuged for 5 min at 5000 rpm. The supernatant was collected and then the tube was blown to near dryness under nitrogen. The pellet was redissolved with methanol–water and the solution was filtered through a 0.22 μm PTFE syringe filter and the filtrate was subjected to instrumental analysis. The mean recoveries were ranged from 68.0 to 120.0%, and the relative standard deviation (RSD) ranged from 0.18 to 6.29%. The linearity ranged from 2.5 to 2000 μg/kg with correlation coefficient greater than 0.99, while limits of detections ranged from 0.05 to 50 μg/kg, and limits of quantification ranged from 0.1 to 200 μg/kg, which were below the legal limits set by the European Union for the legislated mycotoxins. The developed method was applied to 130 corn samples. Among the mycotoxins studied, aflatoxins B1 and fumonisins B1, B2 and B3 were the most predominant mycotoxins, and their concentrations were 0–593.12, 0–2.01 × 104, 0–6.94 × 103 and 0–3.05 × 103 μg/kg, respectively [50].

A sample preparation based on QuEChERS was developed of the analysis of 14 mycotoxins in rice. The method involved mixing 10 g of rice sample with 10 ml of water and 10 ml of acetonitrile containing 10% formic acid. The mixture was then shake with automatic shaker followed by addition of 4 g $MgSO_4$, 1 g sodium citrate tribasic dihydrate and 0.5 g sodium citrate dibasic sesquihydrate and the tube was shaken vigorously with hand for 1 min, and then centrifuged for 5 min at 3400 rpm. The supernatant was transferred to dSPE tube containing 1.2 g of $MgSO_4$, 0.25 g of C18, 0.25 g of aluminum nitride and 0.4 g PSA and the tube was centrifuged at 3400 rpm for 5 min. The supernatant was evaporated to dryness under stream of nitrogen and was reconstituted with 1 ml of mixture of methanol/acetonitrile (1:1 v/v %), vortexed for 1 min and filtered through 0.2 um nylon syringe filter, and the filtrate was analyzed using ultra performance liquid chromatography triple quadruple mass spectrometer (UHPLC–MS/MS). The method validation gave linearity ranging from 10 to 2500 μg/kg with correlation coefficient greater than 0.99. The average recoveries ranged from 70 to 98.5% and RSD less than 7%. The LOQ and LOD were 1.7–50 μg/kg and 0.5–15 μg/kg, respectively. The developed method was validated according to the European Communities 2002/657/EC and SANCO/12495/2011 [51] guidelines and met acceptability criteria in all cases. The performance of the QuEChERS method was compared with the performance of commercial immuneaffinity column (IACs) and the IAC gave comparable performance, but with higher LODs compared to the developed QuEChERS method, but suffered from some limitations, such as lack of sensitivity for some mycotoxins, does not allow multi-analysis and possible cross-reactivity [52].

5. Conclusion

Mycotoxins are secondary fungi metabolites present in foods which can cause adverse effects on humans and animals. Therefore, it is essential to develop a simple,

effective, sensitive and validated analytical method to monitor mycotoxins. Sample preparation is an important step in the analysis of mycotoxins and other contaminants from complex food matrices. And due to the growing demand for high-throughput multiresidue methods (MRM), researchers have developed several easy to perform sample treatment methods, which are rapid and of low cost, require a minimum volume of solvents, provide a high selectivity without complicated clean-up solutions, and allow analysis of broad range of analytes. QuEChERS is fast and simple analytical method which has been developed and optimized for the analysis of a fast and simple analytical method, although several researchers have over the years modified the original QuEChERS technique, which allow multiresidue analysis.

Most of the QuEChERS methods described in this review were couple to liquid chromatography analysis. This is partly due to the great increases in its sensitivity and selectivity, which has led to a significant contribution in qualitative and quantitative determination of mycotoxins in cereals and related foodstuffs. Also, the increasing use of hybrid mass spectrometers, incorporating mass analyzers that are capable of high mass resolution and accurate mass measurements, mitigates some of the problems associated with selectivity and identification. The improvement and upgrading of the available techniques will determine the effectiveness and efficiency of mycotoxins analysis in food matrices.

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