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Analytical Methods

Principal components analysis: An innovative approach to establish interferences in ochratoxin A detection

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ABSTRACT

This work aimed to establish an innovative approach to evaluate the effect of cereals composition on ochratoxin A extraction by multivariate analysis. Principal components analysis was applied to identify the effect of major matrix components on the recovery of ochratoxin A by QuEChERS method using HPTLC and HPLC, and to validate the method for ochratoxin A determination in wheat flour by HPLC. The matrices rice bran, wheat bran and wheat flour were characterized for their physical and chemical attributes. The ochratoxin A recovery in these matrices was highly influenced ($R = 0.99$) by the sugar content of the matrix, while the lipids content showed a minor interference ($R = 0.29$). From these data, the QuEChERS method was standardized for extracting ochratoxin A from flour using 1% ACN:water (2:1) as extraction solvent and dried magnesium sulfate and sodium chloride as salts. The recovery values ranged from 97.6% to 105%. The validated method was applied to evaluate natural occurrence of ochratoxin A in 20 wheat flour samples, which were contaminated with ochratoxin A levels in the range of 0.22–0.85 μg kg^{-1}.

1. Introduction

The determination of trace contaminants in complex matrices such as food often requires extensive extraction and preparation before instrumental analysis. In recent years, researchers have searched analytical methods that enable reliable identification, quantification and detection of trace concentrations at low cost. The QuEChERS method (Quick, Easy, Cheap, Effective, Ruged and Safe) that involves initial extraction with acetonitrile, followed by partition step after the addition of salts and a cleanup step using dispersive solid-phase extraction (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) has been widely explored. This method attracts attention by minimizing the use of solvents, less time of analysis and high recovery of different analytes, including isoflavones (Bustamante-Rangel, Delgado-Zamarreño, Pérez-Martín, & Carabias-Martínez, 2013), herbicides (Li et al., 2013; Mei, Du, & Cen, 2011), pesticides (Tomasini et al., 2012) and mycotoxins in various matrices (Frenich, Romero-Gonzáles, Gómez-Pérez, & Vidal, 2011; Hackbart et al., 2012; Heidtmann-Bemvenuti et al., 2012; Paíga et al., 2012).

The ochratoxin A (OTA) stands out among the mycotoxins, which is produced mainly by different species of *Aspergillus* and *Penicillium* during storage, and is found in various products in different levels. Considering the harmful effects caused by the ingestion of this toxin, OTA contamination has been a concern, with maximum tolerable levels (MTL) established for different foodstuffs (EC, 2011). Moreover, consumers have searched higher quality food, which can only be reached if more sensitive and reliable methods were used to identify different toxic compounds (Ridgway, Lalljie, & Smith, 2007).

QuEChERS method has been evaluated for extraction of ochratoxin A from food matrices, including bread (Paíga et al., 2012), wine (Fernandes, Barros, & Câmara, 2013), popcorn (Ferreira, Fernandes, & Cunha, 2012), rice bran (Hackbart et al., 2012), animal feed and cereal mixtures (Llorent-Martínez, Ortega-Barrales, Córdova, & Ruiz-Medina, 2013), followed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC), or immunoassays. Ochratoxin is often determined by HPLC equipped with a fluorescence detector (FL), which is a sensitive technique and provides more accurate results (Saito, Ikeuchi, & Katoka, 2012).

The inherent characteristics of each matrix used for QuEChERS extraction can contribute positively or negatively to the recovery of mycotoxin from the medium due to the suppressive effect of some compounds on the fluorescence of the analyte. Thus, the use of multivariate techniques such as principal component analysis (PCA) may be suitable to extract mycotoxins from different

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matrices. This analysis is used to explain the covariance structure of the data through a few linear combinations of the original component variables, and to infer conditions for extracting a component from a distinct matrix (Silva, Andrade, Martins, Seabra, & Ferreira, 2006). There is no report in literature regarding the use of PCA to elucidate interferences of mycotoxicological extraction methods. This method of statistical analysis becomes an interesting alternative to the use of experimental design because it is through multivariate that we mathematically understand the key changes in the data, understanding what happens in the results and what factors manipulate to observe the improvement of these.

This study aimed to evaluate the effect of the major components of cereals (rice bran, wheat bran and wheat flour) on OTA extraction using multivariate analysis, and validate QuEChERS method for the determination of OTA in cereals by different chromatographic procedures.

2. Material and methods

2.1. Materials

All solvents used in the study were HPLC grade. Acetonitrile was purchased from Baker. Milli-Q water was obtained from a Milli-Q water purifying system. The standard for ochratoxin A was purchased from Sigma–Aldrich Chemical Company. It was resuspended in benzene:acetic acid (99:1), resulting in the desired concentration for analysis.

2.2. Sampling

Samples of wheat bran and wheat flour were obtained in a local market, while rice bran was provided by a processing industry in Southern Rio Grande. The samples were homogenized, sieved and standardized with a particle size of 0.5 mm prior to analysis.

2.3. Physicochemical characterization

The protein and lipid contents of the grain samples were determined according to the AOAC (2000). Both reducing sugars and total reducing sugars were determined by the 3,5 dinitrosalicylic acid (DNS) method (Miller, 1959).

2.4. QuEChERS method for OTA extraction

The mycotoxin extraction from rice bran (RB), wheat bran (WB) and wheat flour (WF) was performed according to the QuEChERS method adapted by Hackbart et al. (2012). For this purpose, 10 g sample were weighed, and 20 mL water and 20 mL ACN acidified with 1% acetic acid were added. The homogenization was performed on orbital shaker (Tecnal TE 420) for 10 min at 200 rpm, followed by addition of 1.5 g magnesium sulphate and 0.85 g sodium acetate, and stirring for further 10 min at 200 rpm. The material was centrifuged for 15 min at 3220×*g*, and then 0.3 g magnesium sulfate and 0.5 g diatomaceous earth (Celite 545) were added to the supernatant, followed by vortexing for 1 min and centrifugation for 15 min at 3220×*g*. The supernatant was dried at 60 °C and resuspended in 200 μL benzene for high performance thin-layer chromatography (HPTLC) analysis, and in 1000 μL mobile phase for liquid chromatography (HPLC). The percent recovery was determined by fortifying rice bran, wheat bran, and wheat flour with 1 μg g^{-1} OTA.

The validation of OTA extraction by HPLC-FL in wheat flour followed the method described by Paíga et al. (2012). For that, 5 g sample fortified with 5 ng g^{-1} was weighed, and 15 mL ACN and a salt mixture (12.3 g $MgSO_4$, 1.5 g NaCl, 2.1 g sodium citrate dihydrate) was added. The extraction mixture was homogenized by vortexing for 5 min and centrifuged at 3220×*g* for 10 min. The supernatant was dried at 60 °C and resuspended in 1000 μL mobile phase for HPLC analysis.

The following extraction solvents were tested for OTA extraction from wheat flour:ACN, ACN acidified with 1% acetic acid, and ACN:methanol (1:1). From the results, the water addition (0–30%), the extraction time (5 and 10 min) and the salts composition were determined, these being: salt A (12.3 g $MgSO_4{\cdot}7H_2O$ + 1.5 g sodium acetate); salt B (8.18 g $MgSO_4{\cdot}7H_2O$ + 1 g NaCl); salt C (12.3 g $MgSO_4{\cdot}7H_2O$ + 1.5 g NaCl + 2.1 g sodium citrate dihydrate); salt D (4 g $MgSO_4$ dried + 1 g NaCl); salt E (4 g $MgSO_4$ dried); and salt F (8.18 g $MgSO_4{\cdot}7H_2O$). These variables were chosen because they constitute the critical points to recovery the analite of interest.

The matrix effect on OTA determination was assessed by a matrix curve obtained by the addition of increasing concentrations of ochratoxin A varying from 0.1 to 20 ng mL^{-1}, representing the best extraction method. The matrix effect was calculated according to Eq. (1) (Matuszewski, Constanzer, & Chavez-Eng, 2003).

$$ME = (B/A)x100 \quad (1)$$

where:

B – spiked post extraction area,

A – standard area.

The accuracy (recovery) was performed after fortifying the blank of the best extraction method with ochratoxin A standard solution in three concentration levels (3, 5 and 10 times the limit of quantification (LOQ), in triplicate. Fortified and unfortified samples were allowed to stand for 24 h before extraction, protected from light.

To study the repeatability, the extraction was performed by QuEChERS method adapted for different fortification levels, in triplicate, and each level was injected three times. The RSD (%) was calculated from nine determinations.

2.5. Chromatographic analysis of OTA in the extracts

2.5.1. HPTLC

The chromatographic run was performed in high performance plates DC-Xtra Fertgfolien Alugram Sil G using hexane, ethyl acetate and acetic acid (18:4:1.5 v/v/v) as mobile phase (Lin, Zhang, Wang, Wang, & Chen, 1998). After elution, spots were revealed with aluminum chloride in 15% methanol, and dried at 130 °C for 5 min. The characteristic fluorescent spots of mycotoxin were observed under high intensity short/long wave UV light (254 nm and 326 nm).

The OTA quantification was performed by analysis of the images from the UV camara obtained in 16 mega pixels digital camera, using the ImageJ program (http://rsbweb.nih.gov/ij/). A standard curve was constructed with increasing concentrations of ochratoxin A as follows: 30, 50, 100, 150, 200, 250, and 300 ng. The limit of detection (LOD) was determined as the less noticeable fluorescence in the photographic image. The LOQ was calculated from the lowest different OTA concentration measured accurately.

2.5.2. HPLC

The dried extracts were resuspended in 1 mL solvent mixture comprising the mobile phase (50% acetonitrile, and 50% Milli Q water acidified with 1% acetic acid) (Kumar et al., 2012) and injected into the chromatographic system. The run was performed on HPLC consisting of a LC-10 AT pump, a DGU degasser, a CBM-20A controller, a SPD-20A fluorescence detector, a 20 μL 7725i manual injector, a LC Solution-Shimadzu software and a Kromasil C18 5 μm 150 × 4.6 mm column, with handle injector of 20 μL.

The chromatographic run was performed at 35 °C, with a flow rate of 1.0 mL min^{-1} with fluorescence detection (FL) using excitation and emission wavelengths of 333 nm and 460 nm, respectively. Temperature and flow conditions were established in this work, providing a retention time for ochratoxin A of 7.1 min in a total running time of 10 min.

The analytical curve was constructed using standard OTA solution, diluted in the solvent mixture comprising the mobile phase at increasing concentrations (0.1, 1.0, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0 and 50 ng mL^{-1}), in triplicate. Thus, the linearity range, the determination and correlation coefficients, and the LOD and LOQ were determined. To determine LOD and LOQ, OTA diluted solutions were injected in the solvent mixture comprising the mobile phase at decreasing concentrations to obtain a ratio of 3:1 and 10:1 between the analyte peak and the baseline noise, respectively (Ribani, Bottoli, Collins, Jardim, & Melo, 2004).

2.6. Application of QuEChERS method in commercial wheat flour

Twenty wheat flour samples were collected in supermarkets and purchased by different cooperatives. For each sample, 2 packages containing 1 kg each were collected. A wide range of brands was covered to ensure the method applicability regardless the natural variability of the samples available in Southern Brazil. They were stored at room temperature in their original packaging. On the day of the analysis, two packages of each sample were mixed and subsamples were submitted to OTA extraction using the validated method.

2.7. Statistical analysis

Multivariate statistical analysis is a useful technique to identify common patterns in multivariate data sets. PCA is a powerful visualization tool for data evaluation, which can graphically represent intersample and intervariable relationships and provides a way to reduce the dimension of the data. PCA was carried out with Past (folk.uio.no/ohammer/past), and it was performed to access the correlations between the different matrix components and ochratoxin recovery by QuEChERS method (Hackbart et al., 2012) using HPTLC and HPLC.

3. Results and discussion

3.1. Validation of chromatographic methods

Two techniques were used for determination of ochratoxin A in cereals, HPTLC and HPLC. Although less common, the HPTLC technique along with the software ImageJ does not require expensive analytical instruments, thus it is accessible to laboratories with little infrastructure. The photographic imaging decreases the subjective perceptions. Despite the HPLC technique allows detection of lower contamination levels, it requires more sophisticated infrastructure resulting in higher costs for analysis (Valenta, 1998).

Both methods follow the same principle that is the elution of the compound from the liquid mobile phase through a stationary solid phase, which is non-polar in HPLC and moderately polar in HPTLC, differing on the detection method of the mycotoxin.

In HPTLC, after elution the plates were photographed in UV camera (366 nm), and the luminosity was determined by the software ImageJ. In HPLC, the quantification was performed by a fluorescence detector at wavelengths for ochratoxin A ($\lambda_{excitation}$ – 330 nm; $\lambda_{emission}$ – 460 nm), thus increasing sensitivity to the method (Valenta, 1998).

A chromatogram of the fluorescent spots found in HPTLC, corresponding to different OTA concentrations, was generated through the ImageJ software, as shown in Fig. 1.

The performances of both methods are summarized in Table 1. In HPLC, the limit of detection (LOD) was 0.25 ng g^{-1} and the limit of quantification (LOQ) was 0.50 ng g^{-1} (Table 1). Linear regression analysis suggested that the method is linear (R^2: 0.9985) in the range of 0.1–20 ng mL^{-1}. With respect to HPTLC, the LOD and LOQ were higher as compared to HPLC, with values of 0.3 μg g^{-1} and 0.9 μg g^{-1} for LOD and LOQ, respectively. Linearity was obtained between 50 and 300 ng 15 μL^{-1} (R: 0.9970). The ImageJ software proved to be an efficient tool for reliable quantitative determination of ochratoxin A by HPTLC, as reported by Hoeltz, Monezzi, Manfroi, Noll, and Dottori (2012).

Both detection methods showed a significantly higher correlation, with a coefficient of correlation (R) equals to 0.99. However, with respect to the limits of detection and quantification, the HPLC technique, as expected, showed lower values, and thus met the requirements for low detection limits, being more efficient especially on mycotoxicological degradation studies.

3.2. Effect of matrix components on OTA determination

The QuEChERS method of the present study comes from the original method reported by Anastassiades et al. (2003) and adapted by Hackbart et al. (2012) for whole rice bran, proving the need to study the optimal conditions to provide simplicity and speed to the extraction, besides good applicability, high recovery and selectivity (Prestes, Friggi, Adaime, & Zanella, 2009).

Literature has reported the effects of different parameters on the QuEChERS method, such as solvents, particle size, clean up steps, salts addition, and acidification of the medium (Anastassiades et al., 2003; Prestes et al., 2009). However, there are no experimental reports that demonstrate the matrix effect on the extraction of ochratoxin A by this methodology, necessitating a complete readjustment for each matrix.

Due to the susceptibility of different matrices to contamination by ochratoxin A (cereals, meat, spices, beverages, etc.) it is fundamental to study the main components present in these matrices that can affect positively or negatively the extraction and detection of mycotoxin, avoiding the exhaustive readjustment of the method for each matrix, once changes can be done according to the presence or absence of the component of interest.

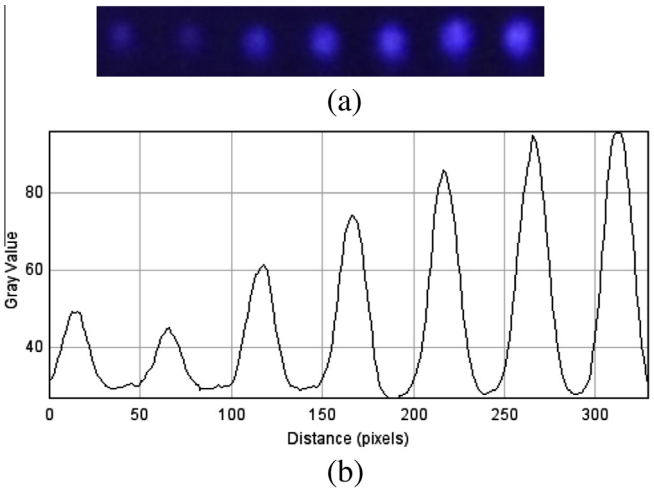

Fig. 1. (a) Picture of the chromatographic plate with increasing concentrations of ochratoxin A, (b) chromatogram obtained of the image of the chromatographic plate.

Table 1
Parameters for validation QuEChERS method.

Parameter	HPLC	TLC
Curve equation	$y = 9151.921x + 7663.58$	$y = 0.351x + 42.54$
Linearity	0.1–20 ng mL^{-1}	50–300 ng
Coefficient of correlation	0.9985	0.9970
Coefficient of determination	0.9971	0.9940
LOD_i*	0.05 ng mL^{-1}	10 ng
LOQ_i*	0.10 ng mL^{-1}	30 ng
LOD_m*	0.25 ng g^{-1}	0.3 μg g^{-1}
LOQ_m*	0.50 ng g^{-1}	0.9 μg g^{-1}

* LOD_i – the detection limit of the instrument; LOQ_i – quantification limit of the instrument; LOD_m – limit of detection; LOQ_m – limit of quantification of the method.

Table 2 shows the chemical and physical characteristics of the grains studied, as well as the percent recovery found by the QuEChERS method in HPTLC and HPLC.

As expected, the OTA determination by HPLC was the most affected by the composition of the cereals fractions, although HPTLC technique found an overestimated value of the contaminant. Wheat bran with higher total sugar content showed lower recovery for all cases.

In this study, multivariate statistics (PCA) was used to prove experimentally the effect of the matrix components on the recovery of ochratoxin A in cereals by QuEChERS modified method (Hackbart et al., 2012) to adjust the extraction conditions.

The use of C_{18} columns in the clean up step of QuEChERS method has been reported for samples with fat content $\geqslant$2%, e.g., rice, barley, wheat and vegetable oils due to the fat content of some portions (Table 2), especially the rice bran of the present study.

However, this procedure was not effective for the other sampling types since the efficiency was greater than 100% in HPLC, and below 70% in HPTLC. Thus, to identify the factor responsible for the response, the results in Table 2 were subjected PCA as shown in Fig. 2.

The PCA is based on the generation of new set of variables, called principal components to explain the variability of results, besides showing the correlation between the different variables. In this study, the variability of the results was explained by two components: component 1 explaining 77.8%, and component 2 explaining 22.2%.

The components graph shows an inverse relationship between samples WF and WB, with the highest and lowest recovery values of OTA, respectively (Fig. 1). With respect to the lipids, proteins, reducing sugar, total sugar, and percent recovery, it is observed that lipids had the least influence on the recovery of ochratoxin A, with a correlation of 0.29 in HPTLC and 0.12 in HPLC. These findings corroborate the studies reported by Prestes et al. (2009), who found that the acetonitrile allows the extraction of minor amounts of co-lipophilic extractives from the sample. The C18 column in the clean up step as described earlier has also limited the effect of the lipids on OTA recovery (Anastassiades et al., 2003).

Both the reducing sugars (RS) and total sugars (TS) were highly correlated (0.99), an expected behavior once the methodology focused on the reducing power before and after carbohydrates hydrolysis. Both RS and TS showed a correlation of −0.99 for OTA recovery by TLC, and −0.98 by HPLC. This interference may have occurred because more polar compounds like sugars are extracted in greater amounts when NaCl is not used in the extraction process and hence there is a greater interference in the detection of the analyte under study (Anastassiades et al., 2003).

Among the variables, lipids showed higher correlation with the component 2, ($R = 0.96$), whereas protein, RS, TS and recovery showed higher correlation with the component 1, with R values of 0.79, 0.99, 0.99 and −0.99, respectively. Thus, probably the component 1 is related to the polar interferences, while the component 2 is related to non-polar compounds.

In general, both extraction techniques are affected by the matrix components in a similar way and with the same intensity, evidencing the applicability of both chromatographic methods, with a correlation of 0.98 between them. Additionally, the polar factors most affected the OTA extraction by the QuEChERS method, indicating that the salt composition in partition must be investigated for standardization of various matrixes.

The multivariate technique allowed to identify the main interference in the OTA extraction and make changes in methodology for each matrix, which could not be done in univariate statistical techniques.

Given that the contamination by ochratoxin A occurs mainly during storage and wheat flour based products are widely consumed, this matrix was chosen for standardization and validation of the chromatographic method for detection of trace amounts by HPLC-FL. Moreover, the wheat flour had the lowest concentrations of reducing sugars and total sugars, with OTA recovery strongly affected by these matrix components.

3.3. Standardizing OTA extraction from wheat flour

The OTA extraction can be affected by various factors, including solvent type, extraction time, salts composition, and type of matrix used. Among the solvents evaluated (Fig. 3a), the acidified 1% ACN increased by twice the OTA recovery as compared to ACN, whereas the mixture ACN:methanol (1:1) did not allow extraction. Frenich et al. (2011) found an increased recovery by acidification of the medium during the OTA extraction from eggs. This increase may be related to the increased stability of the mycotoxin due to acidification of the extraction medium (Prestes et al., 2009).

The addition of water has been studied to improve hydration of the tissue, facilitating the migration of the extraction solvent into the matrix (Fig. 3b). The recovery results ranged from 22% to 66%. The addition of water caused a mean increase of 2.3 times in the time period, and the increase in the extraction time from 5 to 10 min increased 46% mycotoxin recovery with 30% of water addition. This is due to the extracting power of this solvent may be negatively affected by its affinity for sugars from the flour. Therefore, the extraction solvent was standardized as 1% ACN:H_20 (70:30). The present study did not investigate periods longer than 10 min because it would limit the applicability of the extraction and higher water contents could hinder the separation of the aqueous phase from the organic phase.

Table 2
Recovery of ochratoxin A by TLC and characterization of the matrices.

Matrix	Lipids (g 100 g^{-1})	Protein (g 100 g^{-1})	RS* (mg g^{-1})	TS* (mg g^{-1})	Recovery TLC (%)	Recovery HPLC (%)
RB*	19.6 (3.8)	15.9 (3.0)	1.9 (1.4)	8.2 (9.0)	102 (2.4)	51 (1.0)
WB*	4.3 (2.2)	15.6 (4.0)	2.9 (0.6)	15.8 (1.0)	86 (2.9)	43 (2.0)
WF*	7.4 (4.4)	11.7 (2.6)	1.3 (2.7)	4.0 (0.5)	113 (5.5)	61 (3.4)

* RB – rice bran; WB – wheat bran; WF – wheat flour; RS – reducing sugar; TS – total sugar; mean (CV). $n = 3$.

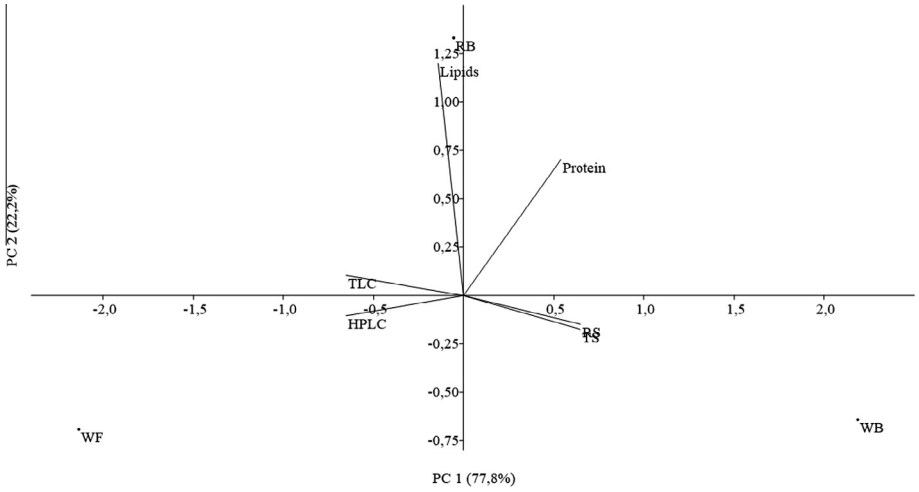

Fig. 2. Effect of matrix components on QuEChERS extraction on TLC and HPLC.

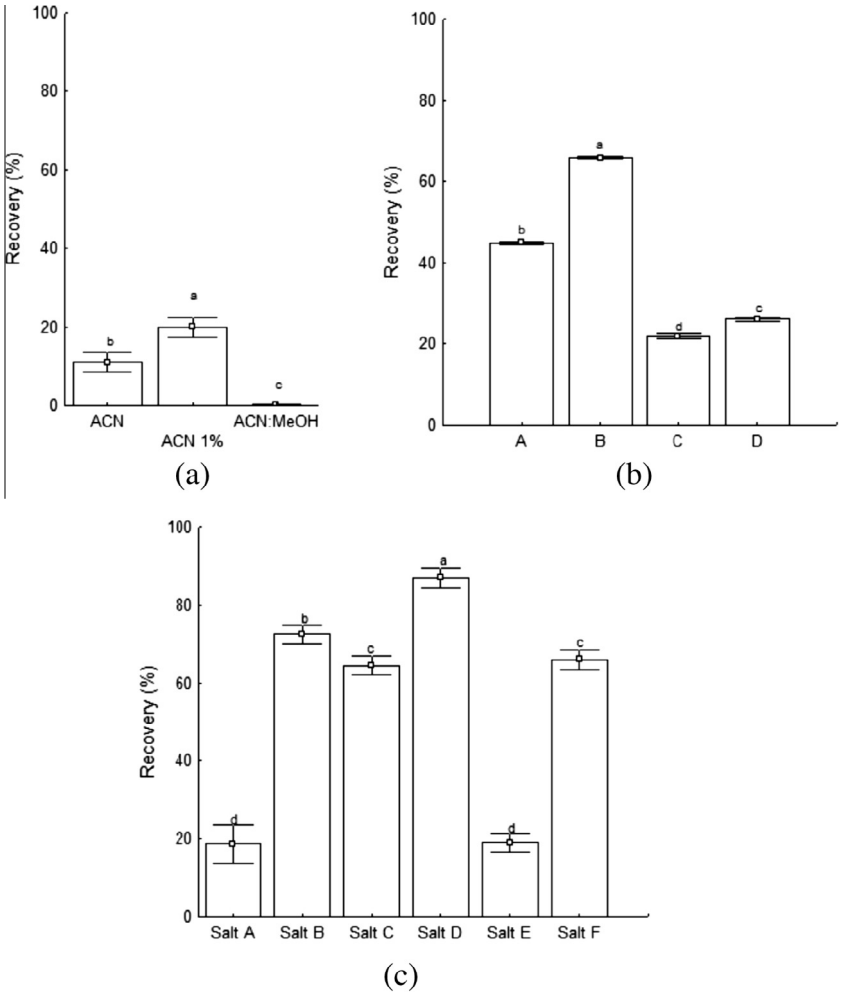

Fig. 3. Effect of solvent (a); water addition and extraction time (b) and different salts (c) on the OTA extraction from wheat flour (ACN – acetonitrile; 1% ACN – acetonitrile acidified with 1% acetic acid; ACN:MeOH – acetonitrile:methanol; A – 30% water/5 min, B – 30% water/10 min; C – 0% water/5 min; D – 0% water/10 min. Salt A (12.3 g $MgSO_4 \cdot 7H_2O$ + 1.5 g sodium acetate); Salt B (8.18 g $MgSO_4 \cdot 7H_2O$ + 1 g NaCl); Salt C (12.3 g $MgSO_4 \cdot 7H_2O$ + 1.5 g NaCl + 2.1 g sodium citrate dihydrate); Salt D (4 g dried $MgSO_4$ + 1 g NaCl); Salt E (4 g dried $MgSO_4$); Salt F (8.18 g $MgSO_4 \cdot 7H_2O$); Bars with identical letters have no significantly different at $p < 0.05$).

In acetonitrile-based extraction methods, the salt addition is very convenient since it is quick, easy, has low cost, with no need to dilute the sample extract, besides providing the separation of the organic and aqueous phases. Moreover, as found in the initial stage of this study, the salt composition is important to eliminate the interferences. Therefore, different salt combinations were evaluated by different QuEChERS methodologies (Fig. 3c).

Table 3
Natural occurrence of OTA in wheat flours.

Sample number	OTA concentration (μg kg^{-1})	%RSD
1	0.31	4.50
2	0.44	9.60
3	0.34	0.60
4	0.46	16.00
5	0.66	6.40
6	0.22	6.30
7	0.32	1.30
8	0.25	12.50
9	0.25	10.30
10	0.26	7.20
11	0.47	7.20
12	0.23	7.50
13	0.34	3.70
14	0.35	6.50
15	0.23	5.70
16	0.85	10.90
17	0.46	8.40
18	0.50	7.80
19	0.24	3.20
20	0.30	2.80

All salts had magnesium sulfate in its composition because of its high capacity for water removal (Prestes et al., 2009). The percent recovery ranged from 18% to 87%, with the best result for salt D, which corresponds to the original QuEChERS method. The magnesium sulfate was dried at 500 °C for 5 h, as recommended by Anastassiades et al. (2003) to eliminate interferences, as phthalates. The drying process provided a 1.2-fold increase in the OTA recovery, due to elimination of interferences, besides avoiding the exothermic reaction occurring in the hydration of magnesium sulfate (Prestes et al., 2009).

The presence of NaCl in the salt composition (when comparing salt E and D) caused an increase of 4.5 times in the mycotoxin recovery. As previously described, it evidences the importance of NaCl in the QuEChERS method due to the fact that polar compounds can be the greatest extraction interferences in wheat flour and other cereals.

The modified extraction method for wheat flour with 87% recovery was defined as follows: 5 g wheat flour and 15 mL 1% ACN:water (2:1), followed by addition of dried magnesium sulfate and sodium chloride and agitation for 10 min under vortexing. Despite the recovery value was within the acceptable limits (70–120%), the matrix effect on the extraction of ochratoxin A was evaluated, since the lowest recovery by HPLC as compared to TLC in the first stage of the present study indicated the depression effect of the signal of compounds in cereals.

The matrix effect (ME) is a study of selectivity aimed to investigate possible interferences caused by substances from the matrix, reducing (ME < 100%) or increasing (ME > 100%) the instrumental signal or instrumental response (Kruve, Kunnapas, Herodes, & Leito, 2008).

For the wheat flour concentrations studied, the matrix effect ranged from 15% to 50%. Due to this high effect on mycotoxins determination, the matrix curve was performed at the same concentrations of the solvent curve, showing linearity between 0.04 and 8 ng g^{-1} and a correlation coefficient of 0.9968. To evaluate the accuracy of the method, calculations were performed with the matrix curve to compensate this effect. The method recovery was 98.7% (3× LQ), 97.6% (5× LQ) and 105% (10× LQ) with a RSD of 11%, 15%, and 6%, respectively.

3.4. Sample analysis

The validated method was applied to the wheat flour samples. From the samples studied, 100% were contaminated by OTA (Table 3). Of these, 85% presented OTA concentrations varying from 0.2 to 0.5 μg kg^{-1}, while 15% of the samples presented OTA ranging from 0.5 to 1 μg kg^{-1}, all results below the limit established by the Brazilian legislation (RDC No. 7/2011) and the European Union (EC, 2011).The validated method demonstrated to be satisfactory, with a RSD lower than 20% for all samples.

Khorasgani, Behfar, and Hydari (2012) evaluated the natural occurrence of OTA in wheat flour, and found 94% of the samples containing OTA levels of 0.004–0.809 μg kg^{-1}. It is worth mentioning that despite the contamination levels are below the legislated value, the chronic action of the contaminant is a challenge for public health authorities due to the high intake of wheat flour based products, as it significantly hinders the adoption of strategies to reduce the long-term effects on population's health.

4. Conclusion

The multivariate technique allowed elucidating the main interference (polar compounds) on the determination of ochratoxin A by QuEChERS method, by both HPTLC and HPLC techniques. Thus, the validated method by HPLC-FL was efficient for wheat flour, with percent recovery ranging from 97.6% to 105%.

5. Conflict of interest

No conflict of interest exists in this research.

References

Anastassiades, M., Lehotay, S., Stajnbaher, D., & Schenck, F. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

AOAC (2000). Official Methods of Analysis of AOAC International. *AOAC International* (17th ed.) (Vols. 1–2). Gaitherburg, Maryland, USA Washington, DC: Association of Analytical Chemists.

Bustamante-Rangel, M., Delgado-Zamarreño, M. M., Pérez-Martín, L., & Carabias-Martínez, R. (2013). QuEChERS method for the extraction of isoflavones from soy-based foods before determination by capillary electrophoresis-electrospray ionization-mass spectrometry. *Microchemical Journal, 108*, 203–209.

European Commission. (2011) Method validation and quality control procedures for pestice residues analysis in food and feed. Document SANCO/12495/2011, National Food Administration, Sweden.

Fernandes, P. J., Barros, N., & Câmara, J. S. (2013). A survey of the occurrence of ochratoxin A in Madeira wines based on a modified QuEChERS extraction procedure combined with liquid chromatography–triple quadrupole tandem mass spectrometry. *Food Research International, 54*, 293–301.

Ferreira, I., Fernandes, J. O., & Cunha, S. C. (2012). Optimization and validation of a method based in a QuEChERS procedure and gas chromatography–mass spectrometry for the determination of multi-mycotoxins in popcorn. *Food Control, 27*, 188–193.

Frenich, A. G., Romero-Gonzáles, R., Gómez-Pérez, M. L., & Vidal, J. L. M. (2011). Multi-mycotoxin analysis in eggs using a QuEChERS-based extraction procedure and ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Journal of Chromatography A, 1218*, 4349–4356.

Hackbart, H. C. S., Souza, M. M., Scaglioni, P. T., Primel, E. G., Garda-Buffon, J., & Badiale-Furlong, E. (2012). The QuEChERS method for determination of ochratoxin a and citrinin in rice and rice bran. *Química Nova, 35*, 1733–1737.

Heidtmann-Bemvenuti, R., Hackbart, H. C. S., Souza, M. M., Badiale-Furlong, E., Dors, G. C., & Fagundes, C. A. (2012). Determination of deoxynivalenol and zearalenone in natural and parboiled rice and their fractions using quEChERS and CLAE/UV-FL. *Química Nova, 35*, 1244–1249.

Hoeltz, M., Monezzi, L. P., Manfroi, V., Noll, I. B., & Dottori, H. A. (2012). Ochratoxin A: Quality analysis of Brazilian and imported wines. *Brazilian Journal of Food Technology, 4*, 58–63.

Khorasgani, Z. N., Behfar, A., & Hydari, R. (2012). Occurrence of ochratoxin A in wheat flour factories in Ahvaz city, Iran. *Toxicology Letters, 211*, S97.

Kruve, A., Kunnapas, A., Herodes, K., & Leito, I. (2008). Matrix effects in pesticide multi-residue analysis by liquid chromatography–mass spectrometry. *Journal of Chromatography A, 1187*, 58–66.

Kumar, R., Ansari, K. M., Saxena, N., Dwivedi, P. D., Jain, S. K., & Das, M. (2012). Detection of Ochratoxin A in wheat samples in different regions of India. *Food Control, 26*, 63–67.

Li, N., Lei, L., Zhang, L. N., Wu, S., Ren, R., Wang, Y., Zhang, H., & Yu, A. (2013). A modified QuEChERS method for the determination of some herbicides in yogurt and milk by high performance liquid chromatography. *Talanta, 105*, 219–228.

Lin, L., Zhang, J., Wang, P., Wang, Y., & Chen, J. (1998). Thin-layer chromatography of mycotoxins and comparison with other chromatographic methods. *Journal of Chromatography A, 815*, 3–20.

Llorent-Martínez, E. J., Ortega-Barrales, P., Córdova, M. L. F., & Ruiz-Medina, A. (2013). Quantitation of ochratoxin a in cereals and feedstuff using sequential injection analysis with luminescence detection. *Food Control, 30*, 379–385.

Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Analytical Chemistry, 75*, 3019–3030.

Mei, M., Du, Z. X., & Cen, Y. (2011). QuEChERS-ultra-performance liquid chromatography tandem mass spectrometry for determination of five currently used herbicides. *Chinese Journal of Analytical Chemistry, 39*, 1659–1664.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426–429.

Paíga, P., Morais, S., Oliva-Teles, T., Correia, M., Delerue-Matos, C., Duarte, S. C., Pena, A., & Lino, C. M. (2012). Extraction of ochratoxin A in bread samples by the QuEChERS methodology. *Food Chemistry, 135*, 2522–2528.

Prestes, O. D., Friggi, C. A., Adaime, M. B., & Zanella, R. (2009). QuEChERS – um método moderno de preparo de amostra para determinação multirresíduo de pesticidas em alimentos por métodos cromatográficos acoplados à espectrometria de massas. *Química Nova, 32*, 1620–1634.

Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. C. S. F., & Melo, L. F. C. (2004). Validation for chromatographic and electrophoretic methods. *Química Nova, 27*, 771–780.

Ridgway, K., Lalljie, S. P. D., & Smith, R. M. (2007). Sample preparation techniques for the determination of trace residues and contaminants in foods. *Journal of Chromatography A, 1153*, 36–53.

Saito, K., Ikeuchi, R., & Katoka, H. (2012). Determination of ochratoxins in nuts and grain samples by in-tube solid-phase microextraction coupled with liquid chromatography-mass spectrometry. *Journal of Chromatography A, 1220*, 1–6.

Silva, B. M., Andrade, P. B., Martins, R. C., Seabra, R. M., & Ferreira, M. A. (2006). Principal component analysis as tool of characterization of quince (Cydonia oblonga Miller) Jam. *Food Chemistry, 94*, 504–512.

Tomasini, D., Sampaio, M. R. F., Caldas, S. S., Buffon, J. G., Duarte, F. A., & Primel, E. G. (2012). Simultaneous determination of pesticides and 5-hydroxymethylfurfural in honey by the modified QuEChERS method and liquid chromatography coupled to tandem mass spectrometry. *Talanta, 99*, 380–386.

Valenta, H. (1998). Chromatographic methods for the determination of ochratoxin A in animal and human tissues and fluids. *Journal of Chromatography A, 815*, 75–92.