Research Paper

UHPLC-ToF-MS method for determination of multi-mycotoxins in maize: Development and validation

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A B S T R A C T

An Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) method has been developed for determination of nine mycotoxins, namely aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisons (FB1 and FB2) in maize. The method included a two-step extraction with acetonitrile 80% (v/v). After optimization, the analytical method was validated. The different concentrations tested take in account the Maximum Levels (ML) for maize (Commission Regulation EC no. 1881/2006) and good results for repeatability (%RSDr = 15.4%), reproducibility (%RSDR ≤ 15.9%) and recovery (77.8–110.4%, except for AFG2 at 2 µg/kg which presented a recovery of 73.4%) were achieved. These met the performance criteria imposed by Commission Regulation (EC) no. 401/2006. The method was applied to twenty-two samples from Portuguese producers of maize. Fumonisins were the most frequently detected mycotoxins, but the levels do not exceed those imposed by European legislation.

1. Introduction

Maize (Zea mays L.) is a staple food in diet, responsible for proving more than one-third of the calories and proteins in some countries (Chulze, 2010). In 2016 the European Union (EU) production of grain maize and corn-cob-mix was around 21% of the total production of main cereals (301 million tonnes) (Eurostat, 2017). Under a wide range of favourable environmental conditions (relative humidity, oxygen, proper temperature, physical damage and presence of fungal spores) and poor hygienic conditions, some fungi, mainly the genera Aspergillus, Fusarium and Penicillium, can produce secondary metabolites, known as mycotoxins, and contaminate food commodities such as maize, consumed by both humans and animals (Sforza et al., 2006; Abia et al., 2013; Anfossi et al., 2016). Therefore, the factors that affect mycotoxins production and dissemination can be categorized in physical (relative humidity, high temperature, insect’s infestation), chemical (use of fungicides and/or fertilizers) and biological (base on the interaction between colonizing toxigenic fungal species and substrate) (Tola and Kebede, 2016). The contamination of commodities can occur in the different stages from the period before harvest, post harvest, storage, processing and post processing (Zheng et al., 2006). Climatic changes can also influence the occurrence of mycotoxins due to extreme rainfall and drought events which favour formation of deoxynivalenol and fumonisins, respectively (Miller, 2008). Mycotoxins are associated with a vast range of adverse health effects including carcinogenesis (Ostry et al., 2017), mutagenesis (Kim et al., 2016), hepatotoxicity (Li et al., 2018), genotoxicity (Domijan et al., 2015) immunotoxicity (Hueza et al., 2014), cytotoxicity (Malekinjad et al., 2015), nephrotoxicity (Schulz et al., 2018), neurotoxicity (Malekinjad et al., 2015) and estrogenic effects (Fink-Gremmels, 1999; Vejdovszky et al., 2017). Mycotoxins can also induce immunosuppression (Moshenszadeh et al., 2016) or cause mycotoxicoses (Abia et al., 2013; Anfossi et al., 2016; Tola and Kebede, 2016; Zheng et al., 2006; Miller, 2008; Ostry et al., 2017; Kim et al., 2016; Li et al., 2018; Domijan et al., 2015; Hueza et al., 2014; Malekinjad et al., 2015; Schulz et al., 2018; Fink-Gremmels, 1999; Vejdovszky et al., 2017; Moshenszadeh et al., 2016; Andrade et al., 2017) (Abia et al., 2013; Andrade et al., 2017). According to the International Agency for Research on Cancer (IARC), aflatoxins (produced from Aspergillus molds) are included in group 1 (carcinogenic to humans), FB1, FB2 (produced from Fusarium molds) and OTA (produced from Penicillium and Aspergillus molds) are included in the group 2B (possibly carcinogenic to humans) and ZEA (produced from Fusarium molds) and T2 (type A trichothecene,
produced from Fusarium molds) are included in group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 1993a, b). Although those of group 3 are considered not carcinogenic, they can cause other adverse effects. For instance, ZEA can affect reproduction in mammals due to its estrogenic activity. In the past some aflatoxicosis outbreaks were reported (Krishnamachari et al., 1975; Ngindu et al., 1982; CDC, 2004, 2005). Apart from acute episodes, the prolonged exposure to mycotoxins can also increase the risk of other human diseases, such as infectious diseases (Antonissen et al., 2014).

Therefore, the occurrence of mycotoxins is monitored and Maximum Levels (ML) are regulated by different regulatory bodies worldwide to assure food safety (Commission regulation No, 1881/2006; Commission Recommendation, 2013). At a global scale, the Joint Expert Committee on Food Additives (JECFA), a scientific advisory board of World Health Organization (WHO) and Food and Agriculture Organization (FAO) is responsible for the evaluation of risks associated with mycotoxins. In the European Union, the European Food Safety Authority (EFSA) scientifically addresses the issues related with mycotoxins and advises the European Commission. Due to the restrictive legislation of mycotoxins in food, sensitive and precise detection methods are demanded allied with low analysis time. Due to the heterogeneous distribution of mycotoxins in cereals and other commodities, it is of utmost importance to implement correct sampling procedures, to obtain reliable results (Zheng et al., 2006). In the past a vast number of analytical systems have been used to determine mycotoxins in food and feed, from immunochemical-based techniques to chromatographic methods (Turner et al., 2009; Bankole et al., 2010; Huang et al., 2014) (Turner et al., 2009; Bankole et al., 2010; Huang et al., 2014). Nowadays LC-MS is the methods that presents more advantages due to the high selectivity, no need of derivatization, simple sample preparation procedures and no need of clean-up, besides simultaneous quantification of multi-mycotoxins at reasonable low cost. However, it is important to assure that the ionization technique (Electrospray Ionization - ESI, Atmospheric Pressure Chemical Ionization - APCl, Atmospheric Pressure Photoionization -APPI) is constant and able to lower matrix effects and ion suppressions (Sforza et al., 2006).

The main goal of this paper was to develop and validate a multi-mycotoxin UHPLC-ToF-MS method to determine aflatoxins (AFB1, AFB2, AFG1 and AFG2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisins (FB1 and FB2) in maize. Validation was carried out in order to meet the criteria of performance of analytical methods established by Commission Regulation EC no, 401/2006. Results from the analysis of 22 different samples of maize from Portuguese producers were presented and compared with ML according to the Commission Regulation EC no, 1881/2006 (Table S1).

2. Materials and methods

2.1. Chemicals and reagents

Methanol, acetonitrile (both HPLC gradient grade) and formic acid were purchased from Merck (Darmstadt, Germany). Water was purified by Milli-Q plus system from Millipore (Molsheim, France). Mycotoxins standards and internal standard (zearalnone, ZAN) were purchased from Sigma-Aldrich (Madrid, Spain) and were dissolved in acetonitrile (AFB2, AFG1, ZEA, T2 and ZAN), methanol (AFB1, AFG2 and OTA) or acetonitrile:water (50:50, v/v) (FB1 and FB2). Stock solutions were prepared with a concentration of 1 mg/mL, except T2, which presented a concentration of 2.5 mg/mL. AFB1, AFB2, AFG1, AFG2, OTA, ZEA, T2, FB1 and FB2 were supplied from Sigma- Aldrich (Table S2). These stock solutions were subsequently used to prepare different working solutions for calibrations. All standard solutions were stored in amber vials in the dark at -20 °C, for at least 2 years, and before use, they were kept at room temperature for 15 min.

Certified reference materials MA1750-1/C1M and MA1764/C1M from Test Veritas (Padova, Italy) were used to evaluate accuracy of the method.

2.2. Samples and sampling procedure

Twenty-two samples of maize from 2018 harvest were provided by InovMilho (Portuguese National Competence Center for Maize and Sorghum Cultures) for quantification of multi-mycotoxins. These samples were intended for human consumption and were collected from the field experimentation trials located in the Coruche region of Portugal from September to October 2018. Each test portion (5 kg) corresponds to a trial modality and was hand collected after thorough mixing of several incremental samples taken from random field place locations. The laboratory samples have been homogenized by grinding (Retsch rotor mill SK 300 with sieve of trapezoid holes of 1.00 mm) the entire test portion (5 kg) and the flours were mixed thoroughly for guarantee complete homogenisation as possible. From each sample, three sub-samples of 50 g each were placed in separate sterile sample collection tubes and preserved at -20 °C until analysis. No further processing of the samples was done.

2.3. Extraction procedure for UHPLC-ToF-MS

About 2 g of maize flour (2.0 ± 0.1 g) was weighted in 50 ml polypropylene tubes. Internal standard (zearalnone) was added (100 µl from a 10 µg/mL). Subsequently, maize was extracted with 10 mL of acetonitrile 80% (v/v) for 1 h at 110 rpm using a Kottermat 4010 Orbital shaker (Uetze/Hänigsen, Germany). After centrifugation at 3000 rpm for 10 min, the supernatant was removed to another Falcon tube and samples were re-extracted with the same volume of acetonitrile 80% (v/v) for 1 h. After centrifugation (3000 rpm, 10 min), supernatants were collected. For analysis of fumonisins, one mL of the extract was diluted with 1 mL of ultra-pure water, filtered through a PVDF mini-uniprepTM and injected into the UHPLC-ToF-MS system. For the analysis of the other mycotoxins, 8 mL of the extract was transferred to a 15 mL Falcon tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was redissolved with 1 mL of acetonitrile 40% (v/v), vortexed for 30 s, filtered through a PVDF mini-uniprepTM and injected into the UHPLC-ToF-MS system.

2.3.1. Spiking experiment

To determine the recovery of the target analytes, spiking experiments were performed. Calibration standards were prepared by spiking blank sample of maize (2 g) with 6 different concentrations of multi-mycotoxins standard solution prepared in acetonitrile 80% (v/v), thoroughly mixed and kept at ambient temperature in the dark for 30 min. Afterwards extraction was performed as described in sub-Section 2.3.

2.4. LC-ToF-MS parameters

Detection and quantification was performed with a Nexera X2 Shimadzu UHPLC coupled with a 5600+ ToF-MS detector (SCIEX, Foster City, CA) equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI+). In terms of chromatographic conditions a column Zorbax Eclipse Plus C18 (2.1 x 50 mm, 1.8 µm) was used and kept at 30 °C, the autosampler was maintained at 10 °C to refrigerate the samples and a volume of 20 µL of sample extract was injected in the column. The mobile phase consisted of 0.1% formic acid [a] and acetonitrile [B] with a flow rate of 0.5 mL/min and with the following gradient program: 0–12 min from 90% to 30% [A]; 12–13 min from 30% to 10% [A] and kept until 14 min; back to 90% [A] from 15 to 16 min until the end of the run (total of 17 min). In terms of mass spectrometry the acquisition was performed in full-scan from 100 to 750 Da using the Analyst® TF (SCIEX, Foster City, CA) software and with the following settings: ion source voltage of 5500 V; source temperature 575 °C; curtain gas (CUR) 30 psi; Gas 1 and Gas 2 of 55 psi; declustering potential (DP) 100 V. Every 10 injections the ToF-MS detector was calibrated in the mass range of the method, to guarantee the accurate mass resolution.
Fig. 1. Chromatogram of a blank maize sample spiked with 2 μg/kg of AFB1, 4 μg/kg of AFB2, AFG1 and AFG2, 3 μg/kg of OTA, 1000 μg/kg of FB1 and FB2, 200 μg/kg of ZEA and T2.
2.5. Identification of mycotoxins

The identification and data processing were made through the PeakView™ and MultiQuant™ (SCIEX, Foster City, CA) softwares.

In terms of identification criteria three parameters were used: maximum relative retention time deviation ($\Delta$RRT) of 2.5%; difference in the isotope pattern with a tolerance of 10% and exact mass deviation ($\Delta$m) with a tolerance of 5 ppm. The isotope match is presented automatically by the PeakView™ software although for the other criteria the following equations were used:

**Equation (1): Relative Retention Time (RRT)**

$$\text{RRT} = \frac{\text{RT}_{\text{analite}}}{\text{RT}_{\text{internal standard}}}$$

Where $\text{RT}_{\text{analite}}$ is the retention time of the analyte, and the $\text{RT}_{\text{internal standard}}$ is the retention time of the internal standard (zearalanone).

**Equation (2): Deviation of RRT ($\Delta$RRT)**

$$\Delta\text{RRT} \, \% = \left( \frac{\text{RRT}_{\text{spiked samples}} - \text{RRT}_{\text{standard}}}{\text{RRT}_{\text{standard}}} \right) \times 100$$

**Equation (3): Deviation of exact mass ($\Delta$m)**

$$\Delta m \, (\text{ppm}) = \left( \frac{\text{Exact mass} - \text{Detected mass}}{\text{Exact mass}} \right) \times 10^6$$

2.6. Validation of LC–ToF-MS method

The method was validated by the determination of concentration range, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and intra-laboratory reproducibility) and accuracy (using recovery assays).

LOD and LOQ were determined as the concentration that originates a signal-to-noise ratio (S/N) $\geq 3$ and $\geq 10$, respectively. For the determination of repeatability ($\text{RSD}_r$) and intra-laboratory reproducibility ($\text{RSD}_R$), blank samples of maize were spiked at different levels (n=6) take in account the ML of each mycotoxin. In the case of $\text{RSD}_R$ extraction was carried out in different days by different operators.

Precision (repeatability and intra-laboratory reproducibility) and accuracy of the method was evaluated using recovery experiments and certified reference materials.

3. Results and discussion

3.1. Development and validation of UHPLC-ToF-MS method

OTA and ZEA could be studied under positive and negative mode electrospray ionization (ESI+ and ESI-). However, the other studied mycotoxins were determined in ESI+ mode, consequently, this mode was selected in order to determine all the studied mycotoxins. OTA and mainly ZEA presented higher sensitivity in the negative mode (data not shown). Huang et al. (2014), have also reported this for OTA.

Reproducibility was evaluated by the Relative Standard Deviation $\text{RSD}_R$ at 3 different days of analysis, different concentration levels and with different operators and values were considered acceptable. LOD and LOQs are shown in Table 1 and are sensitive enough to meet the requirement imposed by EU regulations for the ML of mycotoxins in maize, except for babyfood. LODs are the same or lower than those reported by Spanjer et al. (2009) in maize slurry, for AFB1 (0.5 μg/kg), AFB2 (1 μg/kg), AFG1 (1 μg/kg), OTA (1 μg/kg), FB1 (100 μg/kg), FB2 (100 μg/kg) and T2 (25 μg/kg). Our results only indicated higher LODs for AFG2 and ZEA.
signal suppression-enhancement (SSE) was used to evaluate matrix effect

The co-elution of matrix components can cause matrix effect and af-

Fumonisins (FB1 and FB2) were the most frequently detected myco-
toxins with 89.3% and 74.6% of the samples respectively. The pres-
ence of these mycotoxins is primarily associated with the growth of the
fungus in cereals and maize [15]. The occurrence of fumonisins in
maize has been reported in various countries, including the United
States, South Africa, and the European Union [16]. The presence of
fumonisins in food can cause adverse health effects, such as myo-
ite, methaemoglobinemia, and cancer [17].

Table 3

Comparison between the assigned contamination levels of the certified reference materials (maize) and the measured value by UHPLC-ToF-MS.

| Certified control material | Mycotoxin | Assigned contamination level (μg/kg) | Satisfactory range (μg/kg) | Measured value (μg/kg) |
|----------------------------|-----------|--------------------------------------|---------------------------|-----------------------|
| MA1750-1/CM                | AFB1      | 9.34                                 | 5.23–13.4                 | 8.66                  |
|                            | AFB2      | 0.42                                 | 0.24–0.60                 | 0.52                  |
|                            | AFG1      | 1.57                                 | 0.88–2.26                 | 1.06                  |
|                            | AFG2      | traces                               |                           |                       |
|                            | AFB1 + AFG1 + AFG2 | 11.5                      | 4.32–18.8                 | 10.2                  |
|                            | FB1       | 2545                                 | 1272–3817                 | 2370                  |
|                            | FB2       | 608                                  | 399–818                   | 482                   |
|                            | FB1 + FB2 | 1714–5143                            |                           | 2852                  |
| MA1764/CM                  | ZEA       | 190                                  | 112–269                   | 199                   |

It was considered signal enhancement, when SSE >100%, inexistence of
matrix effect when SSE =100% and signal suppression when SSE <100%. Signal suppression was found for AFB1 (SSE = 95.2%), AFB2 (87.7%) and AFG2 (87.8%). In the case of ZEA, matrix effect was negli-
gible (101.5%). However, it was found signal enhancement for FB1
(SSE = 121.4%), FB2 (SSE = 129.3%) and T2 (SSE = 112.9%). For OTA
this effect was prominent (194.1%) and it has already been reported
for other authors, for instance Huang et al. (2014) have reported for OTA an
SSE% in the range of 173–177% for raw, liquid and powder milk.

3.2. Occurrence of mycotoxins in maize

FB1 and FB2 were detected in maize samples collected in September–October 2018 (Fig. 2). Table 4 compiles the results of these samples
for FB1 and FB2. All the samples were negative for the other myco-
toxins under study. The values of replicates are shown in order to conclude
about the homogeneity of the sub-samples analysed (n=3). After a

Careful homogenisation process, following the established EU guidelines,
results allow concluding that sub-samples were very homogeneous.
Moreover, any of the samples exceeded EU ML for maize (Table 4).

In the last 2-3 decades, numerous studies have reported Fusarium
infested crops (Placinta et al., 1999; Sulyok et al., 2010; Pereira et al.,
2014). In a study carried out by Doko et al. (1995) different maize gene-
type grown in different countries of Europe and Africa where
compared. At the time, Portugal was indicated as one of the studied
countries with more occurrences of positives (100%) ranging from 90 to
4450 μg/kg. Unfortunately, in this study it was not established the relationship among genotype, area and season.

Abia et al. (2013) have reported the occurrence of mycotoxins in food
commodities from Cameroon. These found a mean concentration of
508 μg/kg for FB1 and 149 μg/kg for FB2 for 37 samples of maize. In a study
published by Soleimany et al. (2012), the levels of FB1 in maize meal from
Malaysian markets were in the range 48.2–209.3 μg/kg while for FB2 was in
the range 58.7–113.5 μg/kg. In 2016, Hove et al. (2016) reported that
95% of the maize (n=95) samples analysed (from Zimbabwe) were posi-
tive for FB1 (mean = 242 μg/kg) and 31% for FB2 (mean = 120 μg/kg). In
Brazil, a recent study reported 100% of maize meal (n=148) for FB1 and
FB2 (62.4–66.274 μg/kg) (Oliveira et al., 2017). High frequency (>81%) of
maize samples, from Côte d’Ivoire, contaminated with FB1 + FB2 was also
reported (Manizan et al., 2018). Shephard et al. (2019) recognized that the
high maize consumption in Eastern Cape Province and other parts of Africa
contributes for the high exposure to fumonisins and the promotion of a
diverse diet can lighten this issue.

4. Concluding remarks

It is of utmost importance to control mycotoxins in food chain due to the
severity of adverse health effects, from toxic acute to chronic, in both
animals and humans. The update of legislation is also important to meet
the advances of high-resolution analytical techniques and to assure the protection of individuals. The analytical UHPLC-ToF-MS method developed and validated in maize is an excellent tool to monitor the levels of mycotoxins in this cereal and its application was demonstrated in real samples. Moreover, future work is important in order to include other mycotoxins in the method (e.g. HT2 toxin and deoxynivalenol) evaluate the types of genotypes of maize that can be resistant to the infection caused by fungi, namely species of Fusarium, in order to prevent the development of mycotoxins. Other mitigation strategies are related with the agronomic practices and treatments during the storage of cereal grains in order to decontaminate them (James and Zikankuba, 2018). Finally, it is greatly recommended the educational intervention, the dissemination of good management practices from the field to the agroindustry maize chain in order to reduce the exposure to food contaminated with mycotoxins.

Conflict of interests

Authors declare there is no conflict of interest.

Table 4
Results of the twenty two samples of maize by UHPLC-ToF-MS for FB1 and FB2.

| Samples | FB1            | FB2            |
|---------|----------------|----------------|
| 1       | <LoQ           | <LoD           |
| 2       | <LoQ           | <LoD           |
| 3       | <LoQ           | <LoD           |
| 4       | <LoD           | <LoD           |
| 5       | 848 ± 65.3     | 196 ± 8.2      |
| 6       | <LoD           | <LoD           |
| 7       | <LoD           | <LoD           |
| 8       | <LoD           | <LoD           |
| 9       | 141 ± 4.1      | <LoQ           |
| 10      | <LoD           | <LoD           |
| 11      | <LoD           | <LoD           |
| 12      | <LoQ           | <LoD           |
| 13      | 446 ± 54.8     | 162 ± 9.7      |
| 14      | 301 ± 42.4     | 102 ± 10.8     |
| 15      | 486 ± 63.8     | <LoQ           |
| 16      | 280 ± 24.2     | <LoQ           |
| 17      | 419 ± 32.1     | 158 ± 14.7     |
| 18      | <LoD           | <LoD           |
| 19      | <LoQ           | <LoD           |
| 20      | 134 ± 17.7     | <LoD           |
| 21      | <LoD           | <LoD           |
| 22      | <LoD           | <LoD           |

LoQ = 125 μg/kg; LoD= 62.5 μg/kg.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfsc.2019.07.001.

References

Abia, W.A., Warth, B., Sulyok, M., Kriska, R., Tchana, A.N., Njobe, P.B., Dutton, M.F., Mouindipa, P.F., 2013. Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). Food Control 31, 438-453.

Andrade, P.D., Dantas, R.R., Loureiro, T., Caldas, E.D., 2017. Determination of multi-mycotoxins in cereals and of total fumonisins in maize products using isotope labeled internal standard and liquid chromatography/tandem mass spectrometry with positive ionization. J. Chromatogr., A 1490, 138-147.

Anfossi, L., Giovannoli, C., Baggiani, C., 2016. Mycotoxin detection. Curr. Opin. Biotechnol. 37, 120-126.

Antonissen, G., Martel, A., Pasmans, F., Ducatelle, R., Verbrugghe, E., Vandembroucke, V., Li, S., Hasebrouck, F., Van Immerseel, F., Croubels, S., 2014. The impact of Fusarium mycotoxins on human and animal host susceptibility to infectious diseases. Toxins 6, 430-452.

Bankole, S.A., Schollenberger, M., Drochner, W., 2010. Survey of ergosterol, zearalenone and trichothecene contamination in maize from Nigeria. J. Food Compos. Anal. 23, 837-842.

CDC (Centers for Disease Control and Prevention). 2004. Outbreak of aflatoxin poisoning-eastern and central provinces, Kenya, 2004. MMWR (Morb. Mortal. Wildy. Rep.) 53, 790–792.

CDC (Centers for Disease Control and Prevention), 2005. Aflatoxicosis Outbreak. Kenya Report to the Kenya Ministry of Health.

Chulze, S.N., 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Addit. Contam. A 27, 651–657.

Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off. J Eur Union 191, 2013, 12–15.

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Euro. Union, L 364, 2006, 5–24.

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off. J. Euro. Union, L 70, 2006, 12–34.

Doko, M.B., Rapior, S., Visconti, A., Schjoth, E., 1995. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. J. Agric. Food Chem. 43, 429-434.

Domijan, A.-M., Gajski, G., Jovanovic, I.N., Geric, M., Garaj-Vrhovac, V., 2015. In vitro genotoxicity of mycotoxins ochratoxin A and fumonisin B1 could be prevented by sodium copper chlorophyllin – implication to their genotoxic mechanism. Food Chem. 170, 450–463.
