Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.35821

Action of phenolic extract obtained from rice bran fermented with *Rhizopus oryzae* in the synthesis of trichothecenes and emerging mycotoxins in sweet corn

Taiana DENARDI-SOUZA[1], Carlos LUZ[2], Jordi MAÑES[2], Eliana BADIALE-FURLONG[1]*, Giuseppe MECA[2]

Abstract

The phenolic extracts obtained from rice bran cultivated with *Rhizopus oryzae* were evaluated about their abitily to inhibit the production of trichotecenes and emerging mycotoxins by *Fusarium* strains on sweet corn cobs (*Zea mays* L.). The rice bran was cultured with *R. oryzae* for 48 h at 30 °C. The phenolic compounds were extracted, clarified, lyophilized and ressuspend as water solution with different concentrations. The phenolic acid extracts were applied on sweet corn inoculated by *F. cerealis, F. graminearum* and *F. poae* incubated by 15 days at 25 °C and afterward the fermented corn was autoclaved and frozen. The mycotoxins were quantified by LC-MS/MS. The mycotoxin production by all Fusarium strains evaluated was reduced in relation to the control. *F. poae* was the most sensitive, because its production of the toxins T-2 and HT-2 and BEA was inhibited by phenolic extract 0,5% (p/v), while extract 1% (p/v) inhibited 96% of the DON production. DON, 3ADON and 15ADON production by *F. graminearum* and *F. cerealis* was also reduced. Therefore, the extract is a promising antimicotoxinogenic against Fusarium toxins production on sweet corn cobs and should be a good alternative to reuse wastes from agroindustry.

Keywords: *Fusarium*; mycotoxins; phenolic compounds; solid state fermentation.

Practical Application: Alternative to inhibit the production of *Fusarium* mycotoxins contributing to the food security.

1 Introduction

Corn is a versatile grain that can be used for human consumption in its raw form or as corn-based products of great economic value (Kathage et al., 2016). However, corn crops are prone to fungal infections in the field, after harvest, and also during storage (Grenier & Oswald, 2011). The genus *Fusarium* is found in soil and in the atmosphere, and it can contaminate corn during field cultivation and may persist through storage (Leslie & Summerell, 2008). Inadequate harvesting methods, drying, handling, storage and poor transport, change micota in the substrate and increase the risk of other mycotoxin production with sinergic effect, increasing the risk of mycotoxin production (Marin et al., 2013).

Among the mycotoxins produced by genus *Fusarium* are: trichothecenes (type A, HT-2 and T-2, and type B toxin, deoxynivalenol, DON, and acetylates); zearalenone (ZEA); fumonisin B1 (FB1) and B2 (FB2). There are also emerging mycotoxins that include enniatins (ENs) and beauvericin (BEA), which were discovered after other *Fusarium* mycotoxins, such as fumonisins and trichothecenes (Krska et al., 2008; Marin et al., 2013; Wokorach et al., 2021). These mycotoxins can be harmful to human and animal health, even after the food has been processed, since most mycotoxins remain stable through the processing operations (Schaarschmidt & Fauhl-Hassek, 2021). They may cause acute toxicity, such as estrogenic effects, as well as inducing long-term consequences, such as carcinogenicity, mutagenicity, teratogenicity or immunotoxicity in animals and humans (Bennett & Klich, 2003).

The application of antifungals, that can reduce the mycotoxin contamination, has been studied; however, it has not always been possible to determine the best agricultural system to use in order to reduce the risk of contamination (Arino et al., 2007; Cirillo et al., 2003; Magkos et al., 2006; Scaglioni et al., 2018). It is important to consider that there are evidences that these antifungal compounds are available in wastes from agroindustry, in special the phenolic family (Denardi-Souza et al., 2018a). Their potential against Fusarium genus could be better studied regard the toxigenic potential manifestation, including the emerging mycotoxins ENs and BEA production. If the phenolic extract proves to be effective in preventing the growth of fungi or inhibiting the production of *Fusarium* toxins, it will also be a good alternative to the reuse of waste, solving some aspects of environmental concerns and food safety.

Therefore, this context shows an alternative for the reuse of agroindustry residues through the recovery of antifungal compounds or mycotoxin inhibitors. In this study phenolic extracts obtained from rice bran cultivated with *Rhizopus oryzae* were evaluated about their abitily to inhibit the production of trichotecenes and emerging mycotoxins by *Fusarium* strains on sweet corn cobs (*Zea mays* L.).

Received 02 June, 2021
Accepted 16 Nov., 2021
[1] *Laboratory for Mycotoxins and Food Science, School of Chemistry and Food, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brasil*
[2] *Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, Universitat de València, Burjassot, Spain*
*Corresponding author: dqmebf@furg.br

2 Materials and methods

2.1 Strains and methodology

Rhizopus oryzae (CCT 7560) a generally recognized as safe (GRAS) fungi specie, was obtained from Fundação André Tosello, Campinas, Brazil. Cultures were kept on an inclined plane at 4 °C on potato dextrose agar (PDA). Spores were scraped with 0.2% Tween 80, yielding an aqueous emulsion. PDA was used for new spore incubations for 7 days at 30 °C in Petri dishes. Spores were then scraped with a Drigalski spatula, counted with a Neubauer chamber (Laboroptik Ltd., Lancing, UK), and used as inoculum.

Strains of *Fusarium poae*, *Fusarium graminearum* and *Fusarium cerealis* were purchased from Colección Espanola de Cultivos Tipo (CECT), University of Valencia, Spain. All strains were maintained in PDA at room temperature (25 °C), and their spores were harvested at the time of analysis.

2.2 Rice bran fermentation

The rice bran used as substrate was supplied by agroindustries from Rio Grande do Sul, Brazil. Fermentation was carried out in tray reactors (12x8x4 cm^3) in rice bran, with standardized granulometry (<0.56 mm) in 2 cm layers, which was autoclaved. The substrate (100 g) was homogenized with 45 mL nutrient solution (2 g/L KH_2PO_4, 1 g/l $MgSO_4$, 8 g/L NH_2CONH_2 in 0.4 N HCl), and the moisture of the system was adjusted to 50% with sterile water before beginning the culture. The inoculation was performed with a *R. oryzae* spore suspension at $4.0x10^6$ spores/g substrate. The reactors were covered with sterile gauze and then incubated at 30 °C for 48 h. After incubation, the biomass was stored at -18 °C until it was extracted for the phenolic compounds (Schmidt et al., 2014).

2.3 Extraction and quantification of phenolic compounds

The phenolic compounds were extracted from rice bran biomass samples using methanol (1:10, m/v), as described by Souza et al. (2011) with modifications. Five milliliters distilled water was added to the samples, which then stood for 30 min. Hexane was added and the samples were homogenized for 30 min on a horizontal shaker. Methanol was added and the stirring was continued. The samples were centrifuged, and the hexane fraction discarded. Methanol present in the extract was evaporated on a rotary evaporator at 50 °C, and the residual aqueous solution was clarified with 5% ZnSO4 and 0.1 M Ba $(OH)_2$ and filtered. The phenolic extract was lyophilized and stored at -18 °C.

The lyophilized extract was resuspended in distilled water and quantified using Folin Ciocalteu reagent at 750 nm and a standard curve of gallic acid (8.5-25.5 µg/mL; (Souza et al., 2011).

2.4 Application in sweet corn

To evaluate the phenolic extracts effect on mycotoxin production they were applied to sweet corn cobs, for which 6 cm of the lower end and 3 cm of the upper end were discarded, resulting in 3 parts of 3 cm each (Figure 1).

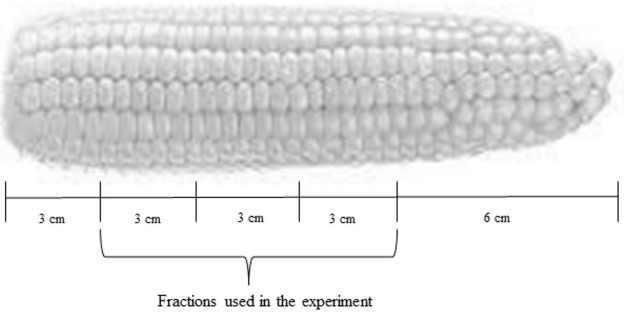

Figure 1. Dimensions of the sweet corn cob used in the experiment.

A stem was fixed to the center of each cob for better handling. With the aid of a manual disperser, the phenolic extract was applied at 0.25%, 0.5% or 1.0% (p/v) as a spray. Corn cob segments were maintained vertically, and after the extracts had dried, spores ($1x10^3$ spores/g) of *F. poae*, *F. graminearium* and *F. cerealis* were added. A control experiment was conducted where the phenolic extract was replaced by sterile water.

The sweet corn cobs were incubated in glass containers covered with plastic film, with holes for air circulation (Figure 2). Incubation was conducted for 15 days at room temperature (25 °C) before the samples were subjected to mycotoxin extraction.

2.5 Extraction of multi-mycotoxins

At the end of the experiment, the sweet corn cobs were autoclaved, the grains were removed and frozen at -8 °C. Still frozen, the kernels were finely ground with an Oster Classic grinder (Newell Brands, Madrid, Spain).

The method for multi-mycotoxins extraction was standardized by Quiles et al. (2016) and Saladino et al (2017). Five grams of each sample was weighed in a 50 mL plastic tube and 25 mL methanol were added. Extraction was performed using a T18 basic Ultra-Turrax (IKA-Werke GmbH & Co., Staufen, Germany) for 3 min. The extract was centrifuged at 4500 x *g* for 5 min at 5 °C and the supernatant was evaporated in a Büchi Rotavapor R-200 (Büchi Labortechnik AG, Postfach, Switzerland). Dried residue was dissolved with 5 mL methanol, that were evaporated under nitrogen at 35 °C using a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA). The residue was resuspended with 1 mL methanol and by vortexing. It was then passed through a 13 mm/0.22 µm nylon filter and subjected to liquid chromatography associated with tandem mass spectrometry (LC-MS/MS). To monitor the changes caused by fermentation, an extraction in maize kept under the same conditions without inoculation of fungi was used. All extractions were performed in triplicate.

2.6 Determination of mycotoxins

A liquid chromatograph 1200 (Agilent Technologies, Palo Alto, CA, USA) with an LC-20AD binary pump, a homoeothermic SIL-20AC, self-sampler and a CMB-20 controller was used. Software Analyst 1.5.2 was employed for data acquisition and processing. Separation of mycotoxins was performed on a Gemini

Figure 2. Illustration of the application of phenolic extract, obtained from rice bran cultivated with *R. oryzae,* to sweet corn exposed to different strains of *Fusarium*.

NX C18 column (150 × 2.0 mm I.D, 3.0 µm; Phenomenex, Palo Alto, CA, USA) at room temperature (20 °C). The mobile phase was composed of solvents A (5 mM ammonium formate and 0.1% formic acid in water) and B (5 mM ammonium formate and 0.1% formic acid in methanol) at a flow rate of 0.25 mL/min. The elution gradient was initially established with 10% eluent B, increased to 80% over 1.5 min, remaining constant for 1.5-4 min, increased to 90% for 4-10 min, increased again to 100% for 10-14 min, and finally returned to the initial conditions and re-equilibrated for 10 min. The injection volume was 20 mL.

A 3200QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) was used equipped with a positive mode ESI interface for detection in multiple reaction monitoring (MRM). The main parameters of MS were optimized as follows: gas nebulizer (GS1), 55 psi; auxiliary gas (GS2), 50 psi; curtain gas (CUR) 15 psi; capillary temperature 550 °C; ion Spray Voltage (IS) 5500 V. Nitrogen was used as the nebulizer, heater, curtain and collision gas (Quiles et al., 2016). The precursor product item transitions were: m/z 801.2/784.1 and 244.1 for BEA; m/z 657.3/196.1 and 214.0 for ENB; m/z 671.2/214.2 and 228.1 for ENB1; m/z 699.4/210.2 and 228.2 for ENA; m/z 685.4/214.2 and 210.2 for ENA1; m/z 722.4/334.3 and 352.3 for FB1; m/z 706.4/336.2 and 318.3 for FB2; m/z 297/161, 203, 231 and 249 for DON; m/z 484.3/185.1 and 215.1 for T-2 toxin; m/z 484.3/185.1 and 215.1 for HT-2 toxin; m/z 339/203.1 and 231.0 for 3ADON toxin; and m/z 339/321.0, 161.0 and 231.0 for 15ADON toxin.

2.7 Statistical analysis

Analysis of variance (ANOVA) and Tukey's test were conducted using the Statistica 6.0 software (Dell, Round Rock, TX, USA) to evaluate for statistical differences ($p<0.05$) of the concentration of phenolic extract regard to mycotoxins produced by each strain studied.

3 Results and discussion

Solid state fermentation of *R. oryzae* in rice bran was able to increase the content of phenolic compounds by 110% (Denardi-Souza et al., 2019). The content of total phenolic compounds in the biomass after the fermentation process was 38.35 mg gallic acid/g. In this study, a total of 18 compounds were identified in rice bran cultivated with *R. oryzae*. Identification was performed based on the relative retention time and mass spectra obtained using quadrupole time of flight LC-QTOF-MS (Denardi-Souza et al., 2018b).

As this is an in vivo experiment, a screening of mycotoxins that were synthesized by different strains of *Fusarium* during the period of maize cultivation was carried out. It is known that this is a very receptive matrix for these fungi and that the synthesis of mycotoxins is a biochemical process, not always linear. The chromatographic method applied to the study was described and validated by Quiles et al. (2016) and Saladino et al. (2017), for the quantification of mycotoxins in corn a white experiment (without strain) was carried out, minimizing possible matrix effects.

In the control experiment with *F. poae*, DON, T-2 toxin, HT-2 toxin and BEA were detected (Table 1).

For DON production, treatment with 0.25% phenolic extract showed a reduction of 88%, and with 1.0% phenolic extract the reduction was 96%. Application of phenolic extract reduced the production of T-2 toxin (62%) present in the treated sweet corn; however, it produced an increase in HT-2 toxin content (74%). Part of this increase might be derived from the conversion of T-2 toxin into HT-2 when the phenolic extract was at a concentration of 0.25%. The production of HT-2 and T-2 toxins presented an inverse behavior suggesting the presence of defense mechanisms in the grain causing detoxification (Desjardins et al., 1993; Food and Agriculture Organization, 2001). However, the toxicities of T-2 and HT-2 toxins are quite similar because they both contain a sesquiterpenoid epoxy moiety (Busman et al., 2011). Toxin T-2 and HT-2 were not detected when concentrations of 0.5% and 1.0% of extract were applied to sweet corn. BEA was only detected in the control experiment.

F. graminearum released DON, 3ADON and 15ADON in the control samples and in all the treatments. When the sweet corn was treated with the 1% (p/v) phenolic acid extracts the production of mycotoxins was reduced to 56% for DON, 50% for 3ADON and 36% for 15 ADON. The same treatment promoted a reduction of around 75% in DON level produced by *F. cerealis* (Table 2). Enniatins (ENB, ENB1, ENA and ENA1) and fumonisins (FB1 and FB2) were not detected in any of the studied strains.

Studies that were conducted in the group showed that phenolic compounds obtained from different matrices have an antifungal capacity against toxigenic strains. Pagnussatt et al. (2014) conducted a similar experiment where the effects of phenolic

Table 1. Mycotoxin content (ng/g) in sweet corn with *Fusarium poae* and treated with phenolic extract obtained from rice cultivated with *R. oryzae* (ng/g).

Experiment	DON	T-2 toxin	HT-2 toxin	BEA
Control	815.96 (±366.81)[a]	548.02 (±91.89)[a]	2862.00 (±450.61)[b]	4.58 (±2.07)
0.25%	97.24 (±12,44)[b]	209.85 (±14.32)[b]	10926.34 (±376.74)[a]	<LOQ
0.5%	90.08 (±43.12)[b]	nd	nd	nd
1.0%	32.57 (±32.57)[b]	nd	nd	nd

(Results ± Standard deviation); nd: not detected; BEA: beauvericin; DON: deoxynivalenol; LOQ: limit of quantification (1 µg/L). Different letters in the same column indicate statistical difference ($p<0.05$).

Table 2. Mycotoxin content (ng/g) in sweet corn with *F. graminearum* and *F. cerealis* and treated with phenolic extract obtained from rice cultivated with *R. oryzae* (ng/g).

	Experiment	DON	3ADON	15ADON
F.graminearum	**Control**	569.80 (±34.15)[a]	131,36 (±13.59)[a]	1545,63(±107.76)[a]
	0.25%	327.23 (±123.58)[b]	114,41 (±14.37)[a]	1385,56 (±103.22)[a]
	0.5%	291.27 (± 12.48)[b]	113,46 (±8.79)[a]	1146,78 (±20.50)[b]
	1.0%	248.20 (±75.69)[b]	65.54 (±15.21)[b]	991,90 (±31.92)[b]
F. cerealis	**Control**	327,21 (±57.03)[a]	nd	nd
	0.25%	171,52 (±29.05)[b]	nd	nd
	0.5%	90,09 (±0.05)[bc]	nd	nd
	1.0%	82,35 (±12.00)[c]	nd	nd

(Results ± Standard deviation); nd: not detected. Different letters in the same column indicate statistical difference ($p<0.05$) for each strain.

acid extracts from the algae *Spirulina* sp. (strain LEB-18) were evaluated against mycotoxin production by *F. graminearum*. The extract mainly composed of gallic acid, significantly reduced fungal growth and reduced the concentration of mycotoxins, with a mean reduction of 68% for deoxynivalenol and nivalenol. Heidtmann-Bemvenuti et al. (2016) evaluated the antifungal activity of natural compounds (γ-orizanol, phenolic extract of neem seeds and rice bran) against 3 toxigenic strains of *F. graminearum* isolated from wheat, rice and barley. Phenolic extracts were more effective than γ-orizanol in inhibiting *F. graminearum*. A correlation between deoxynivalenol levels and expression of the Tri5 gene indicates that the natural compounds could be considered as alternatives to synthetic antifungals (Heidtmann-Bemvenuti et al., 2016). Scaglioni et al. (2018) verified the effects of phenolic extracts obtained from *Spirulina* sp. and *Nannochloropsis* sp. on *F. verticillioides* and its ability to produce mycotoxins. The extracts were able to inhibit infection and fumonisin production *in vitro* and *in vivo* in the field more efficiently than a synthetic fungicide. Therefore, natural phenolic extracts are able to inhibit the potential toxigenicity of *Fusarim* species, as well as those made available with the aid of a fermentation process described in this study.

These evidences, including the one of the current studies, confirmed that DON is the most frequent mycotoxin synthesized by *Fusarium* species. This mycotoxin is a worldwide concern for several cereal crops and their derivatives (Denardi-Souza et al., 2015; Ok et al., 2018; Palacios et al., 2017), especially in corn (Hove et al., 2016; Kos et al., 2017; Setyabudi et al., 2012). On exposure to 1.0% (p/v) phenolic extract, the *F. poae* strain showed the highest DON reduction, followed by *F. cerealis* and *F. graminearium*. The antimycotoxigenic potential of the phenolic extract was effective to mitigate this contamination. Further studies are needed to define the pathways of inhibition of mycotoxins produced by *Fusarium* during treatment with the phenolic extract from rice bran, identify possible conversion to other molecules, and to evaluate a strategy for the application of these extracts. Thus, the antimycotoxigenic potential of the phenolic extract from agroindustrial residues has been shown as a promise to mitigate the worldwide problem of *Fusarium* and its toxins, including the emerging ones.

4 Conclusion

This study demonstrated the antimycotoxigenic potential of a phenolic extract obtained from rice bran cultivated with *R. oryzae*, as an effective strategy against the production of *Fusarium* mycotoxins in sweet corn. The phenolic extract from rice bran applied to sweet corn is capable to reduce DON, 3ADON, 15 ADON, T-2 toxin and BEA release by *Fusarium* strains. *F. poae* was the strain most sensitive to the phenolic extracts, showing a 96% reduction in DON levels. In addition to the reduction in the synthesis of *Fusarium* toxins, this is an innovative proposal for the reuse of agroindustrial residues. Thus, meets the demand for alternative ways to reuse biological wastes by addressing questions of environmental concern and legislation.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Arino, A., Estopanan, G., Juan, T., & Herrera, A. (2007). Estimation of dietary intakes of fumonisins B-1 and B-2 from conventional and organic corn. *Food Control*, 18(9), 1058-1062. http://dx.doi.org/10.1016/j.foodcont.2006.07.002.

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, 16(3), 497-516. http://dx.doi.org/10.1128/CMR.16.3.497-516.2003. PMid:12857779.

Busman, M., Poling, S. M., & Maragos, C. M. (2011). Observation of T-2 toxin and HT-2 toxin glucosides from *Fusarium sporotrichioides* by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). *Toxins*, 3(12), 1554-1568. http://dx.doi.org/10.3390/toxins3121554. PMid:22295176.

Cirillo, T., Ritieni, A., Visone, M., & Cocchieri, R. A. (2003). Evaluation of conventional and organic Italian foodstuffs for deoxinivalenol and fumonisins B1 and B2. *Journal of Agricultural and Food Chemistry*, 51(27), 8128-8131. http://dx.doi.org/10.1021/jf030203h. PMid:14690407.

Denardi-Souza, T., Caldas, S. S., Primel, E. G., & Badiale-Furlong, E. (2015). Exposure to deoxynivalenol, Ht-2 and T-2 toxins by consumption of wheat-based product in southern Brazil. *Food Control*, 50, 789-793. http://dx.doi.org/10.1016/j.foodcont.2014.10.015.

Denardi-Souza, T., Leal, C. A., Massarolo, K. C., & Badiale-Furlong, E. (2019). Profile of phenolic compounds released from rice bran by *Rhizopus oryzae* and *Trichoderma reesei*: their relation with hydrolases activity. *Journal of Food Science*, 84(6), 1382-1389. http://dx.doi.org/10.1111/1750-3841.14646. PMid:31141617.

Denardi-Souza, T., Massarolo, K., Tralamazza, S. M., & Badiale-Furlong, E. (2018a). Monitoring of fungal biomass changed by Rhizopus oryzae in relation to amino acid and essential fatty acids profile in soybean meal, wheat and rice. *CYTA: Journal of Food*, 16(1), 156-164. http://dx.doi.org/10.1080/19476337.2017.1359676.

Denardi-Souza, T., Luz, C., Mañes, J., Badiale-Furlong, E., & Meca, G. (2018b). Antifungal effect of phenolic extract of fermented rice bran with *Rhizopus oryzae* and its potential use in loaf bread shelf life extension. *Journal of the Science of Food and Agriculture*, 98(13), 5011-5018. http://dx.doi.org/10.1002/jsfa.9035. PMid:29602173.

Desjardins, A. E., Hohn, T. M., & McCormick, S. P. (1993). Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. *Microbiological Reviews*, 57(3), 595-604. http://dx.doi.org/10.1128/mr.57.3.595-604.1993. PMid:8246841.

Food and Agriculture Organization – FAO. Joint FAO/WHO Expert Committee on Food Additives – JECFA. (2001). *Safety evaluation of certain mycotoxins in food*. FAO, Rome.

Grenier, B., & Oswald, I. P. (2011). Mycotoxin co-contamination of food and feed: meta-Analysis of publications describing toxicological interactions. *World Mycotoxin Journal*, 4(3), 285-313. http://dx.doi.org/10.3920/WMJ2011.1281.

Heidtmann-Bemvenuti, R., Tralamazza, S. M., Fetter, C., Ferreira, J., Correa, B., & Badiale-Furlong, E. (2016). Effect of natural compounds on *Fusarium graminearum* complex. *Journal of the Science of Food and Agriculture*, 96(12), 3998-4008. http://dx.doi.org/10.1002/jsfa.7591. PMid:26699689.

Hove, M., De Boevre, M., Lachat, C., Jacxsens, L., Nyanga, L. K., & De Saeger, S. (2016). Occurrence and risk of mycotoxins in subsistence farmed maize from Zimbabwe. *Food Control*, 69, 36-44. http://dx.doi.org/10.1016/j.foodcont.2016.04.038.

Kathage, J., Gómez-Barbero, M. & Rodríguez-Cerezo, E. (2016). *Framework for assessing the socio-economic impacts of Bt maize cultivation.* Seville: Joint Research Centre.

Kos, J., Hajnal, E. J., Saric, B., Jovanov, P., Nedeljkovic, N., Milovanovic, I., & Krulj, J. (2017). The influence of climate conditions on the occurrence of deoxynivalenol in maize harvested in Serbia during 2013-2015. *Food Control*, 59, 225-233. http://dx.doi.org/10.1016/j.foodcont.2016.09.022.

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., Macdonald, S., & Crews, C. (2008). Mycotoxin analysis: an update. *Food Additives and Contaminants*, 25(2), 152-163. http://dx.doi.org/10.1080/02652030701765723. PMid:18286405.

Leslie, J. F., & Summerell, B. A. (2008). *The Fusarium laboratory manual*. New York: John Wiley & Sons.

Magkos, F., Arvaniti, F., & Zampelas, A. (2006). Organic food, buying more safety or just peace of mind? A critical review of the literature. *Critical Reviews in Food Science and Nutrition*, 46(1), 23-56. http://dx.doi.org/10.1080/10408690490911846. PMid:16403682.

Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, 60, 218-237. http://dx.doi.org/10.1016/j.fct.2013.07.047. PMid:23907020.

Ok, H. E., Lee, S. Y., & Chun, H. S. (2018). Occurrence and simultaneous determination of nivalenol and deoxynivalenol in rice and bran by HPLC-UV detection and immunoaffinity cleanup. *Food Control*, 59, 225-233. http://dx.doi.org/10.1016/j.foodcont.2017.12.005.

Pagnussatt, F. A., Del Ponte, E., Garda-Buffon, J., & Badiale-Furlong, E. (2014). Inhibition of *Fusarium graminearum* growth and mycotoxin production by phenolic extract from *Spirulina* sp. *Pesticide Biochemistry and Physiology*, 108, 21-26. http://dx.doi.org/10.1016/j.pestbp.2013.11.002. PMid:24485311.

Palacios, S. A., Erazo, J. G., Ciasca, B., Lattanzio, V. M. T., Reynoso, M. M., Farnochi, M. C., & Torres, A. M. (2017). Occurrence of deoxynivalenol and deoxynivalenol-3-glucoside in durum wheat from Argentina. *Food Chemistry*, 230, 728-734. http://dx.doi.org/10.1016/j.foodchem.2017.03.085. PMid:28407973.

Quiles, J. M., Saladino, F., Mañes, J., Fernández-Franzón, M., & Meca, G. (2016). Occurrence of mycotoxins in refrigerated pizza dough and risk assessment of exposure for the Spanish population. *Food and Chemical Toxicology*, 94, 19-24. http://dx.doi.org/10.1016/j.fct.2016.05.011. PMid:27222027.

Saladino, F., Quiles, J. M., Luciano, F. B., Mañes, J., Fernández-Franzón, M., & Meca, G. (2017). Shelf life improvement of the loaf bread using allyl, phenyl and benzyl isothiocyanates against Aspergillus parasiticus. *Lebensmittel-Wissenschaft + Technologie*, 78, 208-214. http://dx.doi.org/10.1016/j.lwt.2016.12.049.

Scaglioni, P. T., Blandino, M., Scarpino, V., Giordano, D., Testa, G., & Badiale-Furlong, E. (2018). Application of Fungicides and microalgal phenolic extracts for the direct control of fumonisin contamination in maize. *Journal of Agricultural and Food Chemistry*, 66(19), 4835-4841. http://dx.doi.org/10.1021/acs.jafc.8b00540. PMid:29701989.

Schaarschmidt, S., & Fauhl-Hassek, C. (2021). The fate of mycotoxins during the primary food processing of maize. *Food Control*, 121, 107651. http://dx.doi.org/10.1016/j.foodcont.2020.107651.

Schmidt, C. G., Gonçalves, L. M., Prietto, L., Hackbart, H. S., & Furlong, E. B. (2014). Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rhizopus oryzae. Food Chemistry*, 146, 371-377. http://dx.doi.org/10.1016/j.foodchem.2013.09.101. PMid:24176356.

Setyabudi, F. M. C. S., Nuryono, N., Wedhastri, S., Mayer, H. K., & Razzazi-Fazeli, E. (2012). Limited survey of deoxynivalenol occurrence in maize kernels and maize-products collected from Indonesian retail market. *Food Control*, 24(1-2), 123-127. http://dx.doi.org/10.1016/j.foodcont.2011.09.013.

Souza, M. M., Prietto, L., Ribeiro, A. C., Denardi-Souza, T., & Badiale-Furlong, E. (2011). Assessment of the antifungal activity of S*pirulina platensis* phenolic extract against *Aspergillus flavus. Ciência e Agrotecnologia*, 35(6), 1050-1058. http://dx.doi.org/10.1590/S1413-70542011000600003.

Wokorach, G., Landschoot, S., Anena, J., Audenaert, K., Echodu, K., & Haesaert, G. (2021). Mycotoxin profile of staple grains in northern Uganda: understanding the level of human exposure and potential risks. *Food Control*, 122, 107813. http://dx.doi.org/10.1016/j.foodcont.2020.107813.