

ICFC 2017



International Conference on Food Contaminants

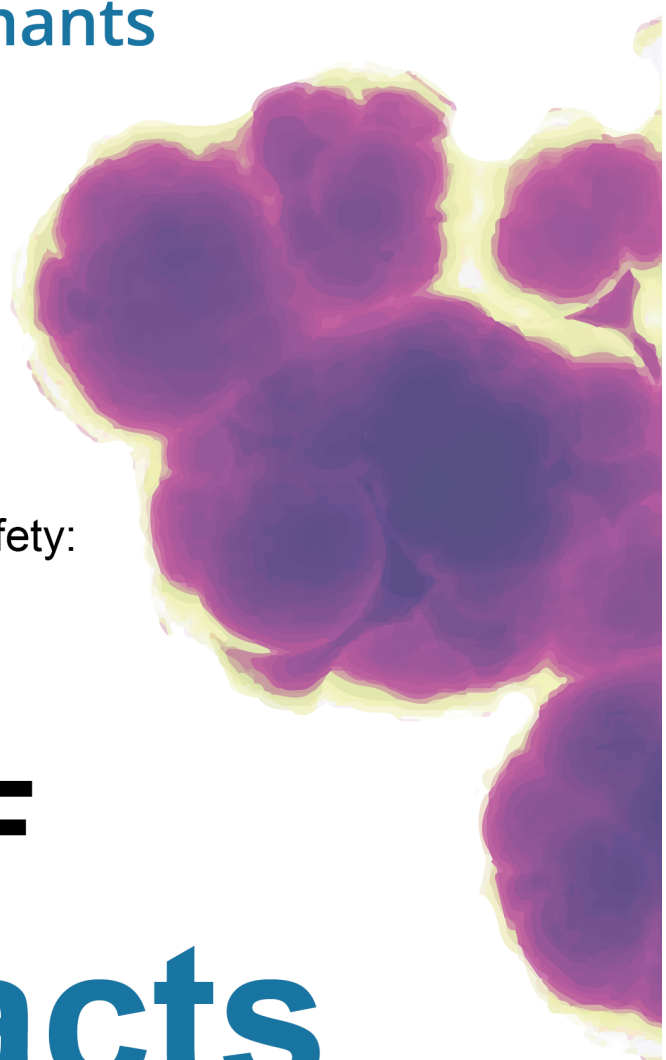
13-14 JULY 2017

BRAGA, PORTUGAL

CONFERENCE THEME

Climate change and food safety:
challenges in the near future

BOOK OF Abstracts



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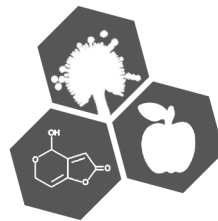
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ICFC2017 Book of Abstracts

Coordinated by

Luís Abrunhosa, Armando Venâncio and Paula Alvito

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The Organization of this event expresses the profoundest gratitude to all those who contributed and supported this event.

This event succeeds another held in Lisbon under the seal of the National Institute of Health Dr Ricardo Jorge (INSA), and, under this same seal, a sequel will be organized in 2019. Therefore, the organization expresses its recognition to the role played by INSA, namely by the Chairman and Vice-Chairman of the Executive Board, Dr Fernando Almeida and Dr José Maria Albuquerque, respectively.

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Dear Participants,

On behalf of the National Institute of Health Dr Ricardo Jorge, we are honoured and delighted to welcome you to the 2nd International Conference on Food Contaminants (ICFC2017) and to receive you in the beautiful city of Braga.

This conference follows the first ICFC, held in Lisbon, in 2015, which focused on the challenges in chemical mixtures.

This second edition of ICFC is dedicated to present and discuss cutting-edge knowledge on climate changes and their impact on food safety issues. As accepted by the general scientific community, the global climate is changing, and Earth is becoming warmer. Climate change is also affecting the occurrence of food safety hazards at various stages of the food chain, from primary production to consumption. There are multiple pathways through which climate-related factors may impact food safety. The rising of CO₂ levels is changing the temperature and precipitation patterns, increasing the frequency and intensity of extreme weather events. These changes are affecting the agricultural and animal production, the dissemination and incidence of crop diseases and pests, and the global food trade, in general.

We hope that ICFC2017 represents an opportunity to network, discuss new ideas and develop future research actions, contributing to reduce the impact of climatic changes on human health and the environment.

We wish you an excellent Conference!

Fernando de Almeida

Chairman of the Executive Board of the National Institute of Health Dr Ricardo Jorge, I.P.



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Ghent

The Local Organizing Committee would like to welcome all of you and express a sincere appreciation for the choice of Braga “city of Baroque”, Portugal, as the place for the 2nd International Food Contaminants Conference. This meeting will be an international forum to gather researchers from around the world, to share and discuss their findings regarding the broad and interdisciplinary field of food contaminants under the theme ***“Climate change and food safety: challenges in the near future”***.

Scientific contributions will cover a variety of topics including climate change and implications for food safety, occurrence and surveillance of chemical contaminants, emerging chemical food contaminants, risk analysis and food safety control systems. Throughout the meeting, we hope to create an atmosphere where everyone, students and professionals, can exchange ideas and establish collaborations.

We look forward to welcoming and hosting you in Braga, one of the most attractive cities in Portugal, and hope this meeting becomes an unforgettable moment.

Luís Abrunhosa, Armando Venâncio and Paula Alvito

Chairs of ICFC2017



ORAL ABSTRACTS



Climate Change and Food Security

Filipe Duarte Santos¹

ABSTRACT

Anthropogenic climate change is one of the more serious environmental challenges of the 21st and following centuries. After a very brief survey of the scientific consensus on the climate change drivers and future projections its impact on food security is analysed. All aspects of food security, including food access, utilization and price stability are potentially affected by climate change. It will be shown that climate change is also likely to increase the risk of food contaminants through the tendency to increase the use of agrochemicals to balance the effects of more frequent extreme weather events and water scarcity in some regions.

1 - Climate Change Impacts, Adaptation and Modelling - CCIAM / cE3c - Centre for Ecology, Evolution and Environmental Changes, FC-UL, University of Lisbon, Portugal

Climate change: implications for marine biotoxinsAna Gago-Martínez¹**ABSTRACT**

Climate change is being an issue of public concern, its impact on various aspects of human and animal health and welfare is a topic widely debated, however the consequences of climate change for the food system (primary production, processing, transport and trading) is not being as deeply studied as other human and animal health and welfare issues. Predictions for climate change in Europe have been evaluated in the EU, in fact the European Commission published a paper on climate change in Europe in 2007, this paper provides projections of climate change in Europe and the influence on areas such as marine areas in which marine biotoxins are included as an important natural contaminant of seafood. The conditions favoring the growth of microalgae producing marine biotoxins include water temperature, sunlight competing microorganisms, nutrients wind and currents directions. A number of these algae produce toxic compounds of different nature which can produce adverse effects not only on other marine organisms but also on human consumers of fish and shellfish containing these toxins. A perceived increase of harmful algal blooms has been globally registered, even if these increase can be associated to tourism expansion, economic exchanges, eutrophication, fisheries activities, etc. the influence of climate changes has been also observed through enhanced and reproduction of dinoflagellates, as well as by the modification of the environment due to changes in the water temperature, salinity, etc. The emergence of toxins in areas where they have not been previously found is widely reported nowadays, an example of this is shown for ciguatera toxins, usually living in tropical and subtropical areas, in fish from French Polynesia, Canary Islands (Spain) Madeira (Portugal), etc., also the recent findings of presence of Tetrodotoxins in mussels from different places in the EU. The presence of these emerging toxins has been associated with the increased temperatures in the waters and therefore attributed to climate change. Reports of the presence of these toxins in European waters, as well as the actions in place will be presented and discussed. Efforts are being made on the impact of these toxins in the EU and risk evaluation and characterization is also being carried out. These will be also summarized and discussed.

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Climate change: modeling for mycotoxinsPaola Battilani¹**ABSTRACT**

The impact and implications of changing climate on food security and quality, consumer habits, trade and economics, regulations and scientific thinking is a hot topic and mycotoxins are main actors. Different biogeography of plants is estimated, fungi (and related toxins) of main concern are expected to change between years and during each year. Overall, climate change is considered to increase health risks, but the conclusion is uncertain in account of many factors involved. Maize in central-southern Europe is an interesting example to stress the system complexity. Maize is commonly contaminated by fumonisins, frequently above the legal limit fixed for human consumption. The first outbreak of severe aflatoxin contamination in southern Europe happened in 2003, followed by points of high contamination in the following years and further serious events in 2012, 2015 and 2016. Then, during the last 13-year period, 2014 showed high deoxynivalenol contamination and 2011 was a safe year, with very low detection of all mycotoxins. In this context of uncertainty, the modelling approach becomes essential. Predictive models allow to generate, in the next or far future, weather data, to draw mycotoxin risk scenarios. Only few examples have been published till now, one with focus on *Aspergillus flavus* in maize at European level. Risk managers, policy makers, Institutions and researchers can receive support from models fed with climate change data to define emerging risks and try to answer to the open question “will mycotoxin contamination be more severe in climate change scenarios?”. Furthermore, predictive models run using actual/historical meteorological data as input became crucial, in this uncertain situation, to highlight risk areas and risk level, and therefore, to support farmers, extension services and stakeholders to rationalize pre- & post-harvest crops and products management. Experiences in modelling applied to mycotoxins and climate change are very limited, but really interesting and useful for developing strategies to better face future risk scenarios.

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Climate changes: challenges in plant toxins

Monique de Nijs,¹ Patrick Mulder,¹ Patricia López Sánchez,¹ Ine van der Fels-Klerx,¹
Leo van Raamsdonk,¹ Hans Mol¹

ABSTRACT

Natural toxins in food, including plant toxins, share one common feature: occurrence and concentration in food is influenced by environmental conditions. Climate is one of the main factors in this respect but the effects of changes in climate on natural toxins in food are not unambiguous for all natural toxins and are poorly understood at this moment. Although current issues with plant toxins in food in the EU are mainly related to agronomic measures, changes in climatic conditions can have a direct effect on contamination of food with plant toxins. The content of inherent plant toxins in food plants, such as in potato and cassava can be altered due to changes in climatic conditions and/or in insect damage. Plant cultivars with more resistance to diseases or drought, which can be related to an elevated level of plant toxins, will be used more frequently, which may possibly result into increased exposure of humans to the inherent plant toxins. Indirect effects occur from co-harvested weeds when weeds spread to geographic areas not infested before. Environmental conditions also influence the occurrence of plant toxins in the co-harvested weeds. Changes in climate are also expected to influence plant diseases and pests and some plants will respond by producing more plant toxins as a defence mechanism. In general, changes in climate share the danger of introducing hazards formerly unknown to the raw material or to the area, which forces governments and food producers to always be aware of natural toxins presence in food and feed. This requires smart and reliable monitoring, early warning systems and modelling tools as well as close cooperation between food and feed producers, governments and scientists. The presentation will focus on possible effects of climate change on occurrence and concentrations of plant toxins in food.

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High throughput methods able to control multiple contaminant classes in a single run

Jana Hajšlová¹, Zbyněk Džuman¹, Milena Stránská¹, Monika Tomaniova¹, Jana Pulkrabová¹

ABSTRACT

Multiple contaminant classes, such as pesticide residues, mycotoxins or plant toxins, may co-occur in food commodities available at the retail market. The assessment health risks associated with such 'cocktail' of potentially hazardous chemicals is rather difficult and are still under investigation. In any case, to protect consumers' health, effective laboratory control is needed. A wide range of methods, nowadays mostly based on high performance liquid chromatography coupled with mass spectrometry (HPLC-MS), with up to hundreds analytes on the target list, has been developed until now for determination of various contaminants originated through similar contamination scenario (agrochemical practices, moulds invasion etc.). Nevertheless, a simultaneous determination of various contaminant groups has been only rarely implemented. The current 'gold' standard in routine laboratories concerned with food safety control is represented by unit resolution tandem mass spectrometric detectors (MS/MS) such as triple quadrupole thanks to high specificity, as well as sensitivity of target analytes detection. On the other hand, due to the monitoring of only specific ion transitions, the key limitation of MS/MS based methods is that, neither post acquisition data re-interrogation, nor screening of unidentified unknowns, is possible. In this context, the growing interest in employing of high-resolution mass analyzers is not surprising. In the recent decade, full-scanning high resolution mass analyzers represented mainly by the time-of-flight (TOF) and orbital ion trap (orbitrap) mass analyzers have offered new challenges in food contaminants analysis. Especially reliable separation of isobaric compounds, thus, enabling reliable analysis of even very complex matrices represents the key benefit. Achievable mass resolution of measurement is a very important parameter related to the mass accuracy obtained. While TOF analyzers available at the market provide mass resolution in order of thousands up to tens of thousands of full width at half maximum (FWHM) units, ultra-high resolution power values higher than hundreds of thousands of FWHM are enabled even by benchtop orbitrap mass spectrometers. Besides the quantitative analysis and targeted screening, identification of 'unknowns' and retrospective data evaluation are the main advantages of these mass analyzers. In our study, we have implemented multi-analyte/multi-group method employing high performance liquid chromatography coupled with tandem high resolution mass spectrometry with Q-orbitrap mass analyser. A comprehensive critical assessment of performance characteristics has been performed. To demonstrate the potential of non-target screening, HR MS/MS spectral library was created using pure standards of more than 350 pesticides, 55 mycotoxins, and 40 plant alkaloids. Validation of the method was realized in QuEChERS-based extracts obtained various food matrices.

Acknowledgments: The financial support of the European Union Horizon 2020 research and innovation programme under grant agreement No 692195 is acknowledged. This work was also supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015).

PortFIR and PT.ON.DATA – integration of Portuguese resources on food contaminants domainRoberto Brazão¹**ABSTRACT**

The PortFIR Programme – Portuguese Food Information Resource – aims the implementation of Portuguese excellence networks in food security and nutrition and, furthermore, the development and maintenance of a platform, with web interface, that includes sustainable databases of recognized quality, transparency and reliability on food composition, contamination and consumption. The Programme, created in 2009, is coordinated by the National Institute of Health Doutor Ricardo Jorge (INSA), in partnership with GS1 Portugal, and collaboration of governmental and private organizations.

Three networks were created up to the present: Food Composition (RPCA), Food Microbiological Information (RPIMA) and Food Chemical Contamination (RPCQA), acting through Working Groups, with the main objectives of update and maintenance of the referred databases, food information management, data analysis and dissemination and knowledge sharing, optimizing the use of national data/information and resources. These databases constitute an important source of data, from multi-origins such as: governmental risk management authorities, universities, research centres, laboratories and food business operators, essential for the update of the Portuguese Food Composition Database (FCDB), the assessment of diet associated risk- benefit and nutritional status of populations and/or other fields of work.

In the food contaminants domain, the major RPCQA network provider will be the National Data Management System "*alimentos PT•ON•DATA*", created in the frame of electronic transmission of analytical data to EFSA, that contains all the national data from the food and feed chain official control plans, covering chemical contaminants, pesticide residues, food additives, biological monitoring and residues of veterinary medicinal products. The *PT•ON•DATA* development was coordinated by INSA and the General Directorate of Food and Veterinary Affairs (DGAV) and involved other National Authorities and Laboratories.

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Influence of raw materials and process variables on 3-Monochloropropane-1,2 Diol (3-MCPD) content in bakery products

Sofia Oliveira ^{1,2}; Marisa Castro ^{2,3}

ABSTRACT

3-Monochloro-1,2-propanediol (3-MCPD) and glycidol are contaminants in processed food, which have been well known for 30 years. The accepted daily intake (*TDI*) for 3-MCPD and its esters was reduced from 2 to 0.8 micrograms per kilogram of body weight per day ($\mu\text{g}/\text{kg bw}/\text{day}$). The present study was focused on identifying the possible causes leading to the level of 3-MCPD found in bakery products in response to changes in the raw materials used and process variables applied. A GC-MS method was developed and applied for 3-MCPD quantification. Two types of derivatizing agents (HFBA and BSTFA) were tested and compared. BSTFA was found to be more appropriate. A calibration was also performed, where a limit of quantification of 1ppb was obtained. In the pilot laboratory, 46 cake recipes from 4 references were analysed to determine the main cause to the levels of 3-MCPD found in the finished product. During these tests, the influence of glycerine, palm oil, salt and chlorine contents, the type of flour and the respective emulsifiers and flavorings were investigated. These variables were selected based on historical data. Dedicated analyses of some major types of raw materials, such as cocoa, were also elaborated. It was concluded that even if a particular raw material is the main cause of the levels of 3-MCPD found in a cake sample, feasible alternatives for replacement may not be possible, since the finished product may not present the quality characteristics expected by the customer. The study in toasts focused on the raw material present in the formulation that most influences the presence of 3-MCPD, the palm oil. Vibrational spectroscopies were used trying to monitor the presence of the contaminants in fat despite the strong chemical resemblance in their chemical structures. In conclusion, factors, such as the presence of chlorine, glycerin, palm oil (or its derivatives) and the high salt content, which can be considered critical, require further investigation.

Keywords: 3-Monochloropropane-1,2 Diol (3-MCPD), bakery products

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Ocean warming and acidification impacts on accumulation and elimination of marine paralytic shellfish toxins in mussels

Ana Catarina Braga^{1,2}; Carolina Camacho^{2,3}; António Marques^{2,3},
Ana Gago-Martínez⁴; Mário Pacheco¹; Pedro Reis Costa^{2,5}

ABSTRACT

Climate is changing, with temperatures rising and the overall ocean pH dropping beyond levels acceptable for many forms of life. The emerging ocean acidification and warming, has potential environmental and ecological implications, with shellfish being subjected to strong selection pressures due to the combined effects of climate change and increasing exposure to toxins derived from harmful algal blooms. The aim of this study was to assess the effects of scenarios of ocean warming and acidification on accumulation and elimination of marine biotoxins in mussels. Mussels were acclimatized during 3 weeks to 4 environmental conditions: 1) a scenario of warming with increased temperature (24 °C), 2) an acidified scenario (pH 7.6), 3) a change of both parameters (24 °C and pH 7.6), and 4) the current conditions (19 °C and pH 8.0). After acclimation, mussels from each treatment were fed with *Gymnodinium catenatum*, a toxigenic dinoflagellate that produces paralytic shellfish poisoning (PSP) toxins, during 5 days and then with non-toxic microalgae *Tetraselmis* sp. during 10 days. Samples were collected for toxin analysis at days 1, 5, 6, 10 and 15, being PSP toxins determined by HPLC-FLD. The highest levels of toxin accumulation were observed in mussels under the current conditions (824 µg STXeq kg⁻¹ at day 5). Although lower toxin levels were accumulated under climate change conditions (24 °C and pH 7.6), longer periods were required for toxins elimination. The profile of toxins in mussels was constituted by N-sulfocarbamoyl (C1+2 and GTX5) and decarbamoyl (dcSTX) in all treatments. In the case of mussels exposed to climate change conditions, increased GTX5 and dcSTX molar fraction was observed at the end of depuration period. In terms of seafood safety, it seems that global changes may lead to lower toxicity levels. However, once mussels are contaminated longer periods are required for PSP toxins elimination.

Keywords: Marine biotoxins, paralytic shellfish poisoning (PSP), *Gymnodinium catenatum*, saxitoxins

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***In vitro* decontamination by inorganic adsorbents of aflatoxin B₁ and Cadmium individually or in mixture**

Chaima Ragoubi¹; Amel Mehrez¹; Aya Ben Amara¹; Insaf Bankaji²; No  mene Sleimi²;
Ahmed Landoulsi¹; Imed Maatouk¹

ABSTRACT

The problem of mycotoxins is a matter of global importance, both economically and in terms of public health, the contamination of food by these contaminants remains difficult to control despite the efforts to limit it. The risks related to mycotoxins could be increasing due to an interaction between these toxins and other contaminants such as heavy metals. Assays to decontaminate these mixtures seems to be critical point. The aims of this work is to investigate the efficacy of some chemical adsorbents (Hectorite, Kaolinite, Sejnein kaolinite and Mg (OH)₂) as a means of decontaminating aflatoxin B₁ (AFB₁) and Cadmium (Cd), separately or in mixture. Different processes have shown an acceptable or even effective decontamination potential. We have tried to determine the ability of these adsorbents to reduce AFB₁ by interaction of mycotoxin at different times of incubation with adsorbents and at different concentrations (adsorbents and AFB₁). The results showed a reduction with all adsorbents with a maximum reduction rate of AFB₁ ranging from of 55% (50 µg) to 95% (20 mg) by kaolinite. Thus, the chemical method seems to be very effective. In addition, we evaluated the efficacy of these adsorbents also for metals (Cd) separately or combined with AFB₁ by a sensitive technique HPLC / FD for mycotoxin analysis and Atomic absorption spectroscopy (AAS) for Cd analysis. The present work has shown the efficacy of these clay minerals as adsorbents of Cd where the percentage of a maximum decontamination of this metal is up to 90% for the Mg (OH)₂. Moreover, the percentage of decontamination of AFB₁ in the presence of Cd ranging from 99% to 100% for some types of clays studied (Hectorite and Kaolinite). The adsorption by the different clays studied appears to be effective for the decontamination of AFB₁ alone or in mixture with Cd and thus could reduce the associated health risks. An application of this decontamination method is promising in food and feed detoxification.

Keywords: Mycotoxins, aflatoxin B₁, decontamination, Cadmium, adsorption, HPLC / FD, AAS

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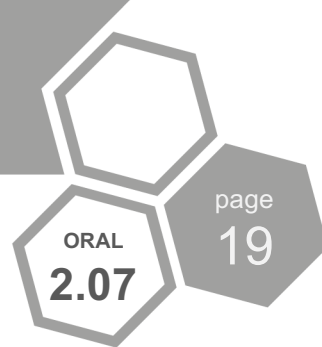
Future challenges to the food safety system

Franz Ulberth ¹, Anne-Katrin Bock ¹, Sandra Caldeira ¹, Kalliopi Mylona ¹,
Petros Maragkoudakis ¹, Jan Wollgast ¹

ABSTRACT

The free movement of safe and wholesome food is an essential aspect of the EU internal market and contributes significantly to the health and well-being of EU citizens and to their social and economic interests. Due to globalisation and the availability of new technological processes the European agri-food sector is becoming more and more complex. The central objective of the EU food policy and legislative framework is the provision of safe, nutritious, high quality and affordable food to Europe's citizens and consumers. Drivers of change (climate change, resource scarcity, technological developments, demographic and societal aspects, global economy and trade) could put the current legislative framework related to the food chain under significant stress and it may be necessary to take measures in order to ensure its future resilience. Scenario building was used to identify potential disruptions and challenges to the current food system and based on this the resilience of the current legislative framework will be tested and research needs identified. Four scenarios were developed using a participatory foresight approach involving stakeholders representing a wide range of interests. The four scenarios represent combinations of the main drivers of change with different weights attached to them. Within the boundaries of this study, the EU legislative framework governing food safety appears to be robust and appropriate. However, certain elements would need to be strengthened to better prepare for future challenges.

Keywords: Food safety, foresight, scenario building

**Food consumption as a contribution to chemical risk assessment**Duarte Torres¹**ABSTRACT**

The chemical risk assessment process, on a food safety perspective, aims to estimate the likelihood of the occurrence of adverse health effects at a given exposure to a chemical hazard. Chemical hazards include environmental contaminants, process derived food contaminants, food contact materials, pesticides, food additives, microbiological or plant derived toxins, or even nutrient compounds from fortified food or food supplements.

During risk assessment, exposure levels are compared with health based guidance values or thresholds of toxicological concern. To assess exposure, i.e. to estimate the likelihood of the consumer being exposed to a substance and to quantify the extent of that exposure is, therefore, a critical element of the risk assessment process. The accuracy of any exposure assessment will ultimately depend on the precision in the two calculation inputs – chemical concentration and food consumption.

The National Food, Nutrition and Physical Activity Survey, 2015-2016 (IAN-AF) aimed to collect representative nationwide and regional data (from 3 months up to 84 years of age) on individual food consumption using an electronic assessment tool for 24-hours recall – the eAT24 module. This module allows to collect food consumption data and to describe consumed foods according to the system FoodEx2. Portion sizes estimation included a digital food picture book, which was developed including 1048 food photos and 39 photos of household measures. Data on concentration of chemicals in foods can be provided by national monitoring and surveillance programs, total diet studies or be estimated from mathematical modelling.

Having food consumption and concentration of chemicals in foods with a common classification and description system, allows a direct match between both datasets for each individual and to estimate the distribution of total exposure, where any percentile of interest can be calculated. The average contribution of each food or food category to the total exposure can also be estimated.

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Contaminants in Portuguese food chain on official control samples. National data management system "alimentos PT•ON•DATA"

Roberto Brazão¹; Francisco Ravaço¹; Sidney Tomé¹; M. Graça Dias¹; Elsa Vasco¹; Sílvia Viegas¹; Paulo Fernandes¹; Patrícia Inácio²

ABSTRACT

Objectives: To gather, harmonize and electronically transmit to EFSA Portuguese analytical data from the food and feed chain official control plans, in the fields of chemical contaminants, pesticide residues, food additives, biological monitoring and residues of veterinary medicinal products, using the National Data Management System (NDMS) "alimentos PT•ON•DATA".

Material & methods: The National Institute of Health Doutor Ricardo Jorge (INSA) and the General Directorate of Food and Veterinary Affairs (DGAV) coordinated the pilot projects contracted with EFSA, for the implementation of the Standard Sample Description - SSD and SSD2, involving other National Authorities and Laboratories, in order to create, develop and upgrade the NDMS. The NDMS was implemented using Microsoft® tools and the SSD's data models and catalogues, the EFSA Guidance on Data Exchange (GDE) and the Food Classification System (FoodEx) as standards.

Results: The creation and upgrade of NDMS allowed Portugal to implement a system with functionalities to upload, map and validate this analytical data and to create XML files, among others, enabling authorities to monitor in real time sampling and execution of the control plans and also to do data reports. Between 2009 and 2015 were generated and reported to EFSA 62257 SSD compliant results, almost entirely of chemical contaminants, and between 2013 and 2015, 190055 results were transmitted according to SSD2, with highest percentage for pesticide residues.

Conclusions: The NDMS implementation allows a continuous gathering and harmonization of data produced and a higher level of automated processes, which contributes to the improvement of its final quality, integrity, and consistency. Furthermore, the system enables an easier and faster data loading, evaluation, utilization and transmission to EFSA, contributing to the optimization of control plans and food chemical and microbiological risk assessment, aiming the improvement of food and feed safety.

Keywords: Contaminants, official control samples, national data management system, alimentos PT•ON•DATA, electronic transmission of analytical data, standard sample description, harmonization



Effect of climate and agronomic practices on mycotoxin contamination in Zimbabwean maize

Melody Ndemera¹; Sofie Landschoot²; Marthe De Boevre¹; Sarah De Saeger¹; Loveness K. Nyanga³

ABSTRACT

The effect of climate on mycotoxin contamination in Zimbabwe was investigated in 3 agro-ecological zones of Zimbabwe. Climatic data, mycotoxin contamination data in maize and the associated agronomic data were collected from 64 households during the 2014/2015 agricultural season. Mycotoxin contamination data were derived from a total of 158 maize samples, collected from the household's harvest at three different time points. Analysis and quantification of mycotoxin contamination in the maize samples was performed using a validated multi-mycotoxin analysis method for 23 mycotoxins. Maize was mainly contaminated by *Fusarium* spp. mycotoxins, namely fumonisin B₁ (FB₁), FB₂, FB₃, nivalenol, de-oxylnivalenol, 15- acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol and zearalenone, occurring in 37, 11, 19, 1, 6, 1, 1,7 and 4% of the samples respectively. Aflatoxin B₁ and sterigmatocystin were found in 1 and 3% of the samples respectively. Mean mycotoxin contamination was below limit of quantification except FB₁ and FB₂, being 193 and 49 µg/kg, respectively. There were no significant differences in mycotoxin contamination in maize across agro-ecological zones. High temperatures, particularly at the flowering stage of the maize, were positively correlated with high levels of FB₁. Rainfall was also positively correlated with FB₁ contamination. Correlations with an absolute value higher than 0.24 were significant at $\alpha = 0.05$, for both temperature and rainfall. The choice of seed and nitrogen fertilizer application were significant ($p = 0.012$ and $p = 0.052$ with regard to seed variety and nitrogen fertilizer application respectively), in modulating FB₁ contamination. There was no significant change in mycotoxin contamination post-harvest. Except for FB₁, good agricultural practices were attributed to low mycotoxin contamination in maize.

Keywords: Mycotoxins, food safety, climate

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Occurrence of mycotoxins in several food matrices from the Spanish market between the years 2001-2016

Ariane Vettorazzi¹; Adela López De Cerain¹; Elena González-Peñas²

ABSTRACT

Compilation of data regarding mycotoxin contamination allows comparison with new data obtained under different climatic conditions, and it is the preliminary step in the study of the influence of climatic change on mycotoxins production. The aim of this study is to present occurrence data of mycotoxins in food matrices in the Spanish market between 2001-2016. Analysis was carried out applying analytical methods validated for each matrix. Ochratoxin A (OTA) in cereals (n=156), beer (n=31) and wine (n=173) as well as 5 OTA-derivatives in wine (n=63) were determined by HPLC-FLD. Patulin in apple juice (n=120) was analyzed with micellar electrokinetic chromatography (MEKC)-UV. HPLC-FLD was used for simultaneous determination of OTA, zearalenone (ZEA) and aflatoxins (AFs) in barley (n=123) and breakfast cereals (n=46). Trichothecenes in barley (n=123) were determined using GC-MS. Finally, 22 mycotoxins were analyzed in 191 milk samples with LC-MS/MS. All samples were obtained from local/national markets or agro-food companies. OTA levels were from <LD (0.013) to 7.61 µg/kg in cereals and cereal-based food and from 0.00049 to 0.32 µg/L in wine. Patulin levels in apple juice were from 0.7 to 118.7 µg/L. Multi-detection methods demonstrated the co-occurrence of more than two mycotoxins in 95% of the barley samples. The most common combinations were AFB₁, OTA and deoxynivalenol (DON) and AFB₁, OTA, DON and ZEA in the 29% and 26% of the samples, respectively. In breakfast cereals, 67% of the samples had detectable levels of AFB₁, OTA or ZEA and 28% of the samples showed co-occurrence of two toxins, mainly OTA and ZEA. In wine, 100% of the samples contained OTA and OTB and in the 75% of the samples co-occurred with OTC. In cow milk no levels of mycotoxins higher than their detection limits were found. Only OTA in cereal-based baby food (10% of samples) and in one corn sample, as well as, patulin in apple juice (11% of samples) exceeded the recommended maximum levels.

Keywords: Mycotoxins, occurrence, Spain, food, multi-detection



Modified (masked) and Emerging mycotoxins

Sarah De Saeger¹

ABSTRACT

The term “masked mycotoxins” was introduced in the early 1990’s by Gareis et al. (1990), with recent literature exclusively referring to them as conjugated mycotoxins generated by plants (Berthiller et al, 2013; Rychlik et al, 2014). Mycotoxins can also be matrix-associated, as well as modified in their chemical structure by mammals, bacteria and fungi, and through food processing. Therefore, the term “modified mycotoxins” has been introduced (Rychlik et al, 2014). Research on modified mycotoxins has gained a lot of global interest and is progressing at a fast pace. The European Food Safety Authority (EFSA, 2014) scientific opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed underlies the attention drawn by risk assessors and policy makers.

The term “emerging mycotoxins” is less well defined. One could adopt the definition of emerging mycotoxins on the base of the definition and description of “emerging risks” of the EFSA (adopted by the Scientific Committee on 10 July 2007): “An emerging risk to human, animal and/or plant health is understood as a risk resulting from a newly identified hazard to which a significant exposure may occur or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard.” Emerging risks will therefore be related to emerging mycotoxins, changing dietary habits, climate change as well as change in mould species.

After a basic introduction, the topic of this presentation will be exemplified by modified *Alternaria* toxins and the emerging mycotoxins beauvericin, enniatins and ergot alkaloids. Moreover, use of high resolution mass spectrometry (HRMS) as the analytical tool for detection and identification of unknown secondary fungal metabolites and modified derivatives will be highlighted with some real-world examples.

Also a call to move beyond the well-studied aflatoxin problem in developing countries will be done during this presentation, while introducing the MYTOX-SOUTH network.

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**Arsenic uptake by plant foods for human nutrition:
current status and new perspectives**

Isabel Castanheira¹; Inês Coelho¹; Sandra Gueifão¹; Marta Ventura¹

ABSTRACT

Arsenic (As), is a metalloid that can accumulate in water, plants and seafood to high levels. Depends on the chemical form it can pose a risk to human health. Inorganic arsenic (Asi) is considered a non-threshold carcinogen, and every exposure constitutes a risk. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) can be related with inorganic exposure and arsenobetaine (AsB) and arsenocholine (AsCh) are organic species with nontoxic effects associated. Speciation data is crucial to clarify the linkage between arsenic content and chronic diseases. A comprehensive dietary exposure assessment is required in particularly to infants and pregnant women. Fetal exposure to inorganic arsenic seems correlated with an increased risk of adverse health effects later in life.

Recent advances in analytical procedures and the metrological principles applied for the identification of arsenic chemical species are discussed. HPLC-ICP-MS as the golden standard technique providing data to support a comprehensive dietary exposure assessment and to redefine risk assessment of (Asi), is presented. The contribution of Total Diet Studies as a harmonised methodology for food sampling and exposure assessment modelling to ensure comparability and consistency of arsenic data across countries is another aim of this presentation. Information about mitigation strategies to reduce exposure to arsenic is also highlighted.

Keywords: Arsenic, speciation analysis, food data, metrology, data exchange

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Connection between mycotoxin contaminations and the development of food allergy

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ABSTRACT

Orally ingested food proteins normally induce oral tolerance, whereas in case of food allergies they cause IgE-mediated allergic sensitization and clinical symptoms varying from mild skin itching to severe and potentially life-threatening anaphylaxis. The initial mechanisms of sensitization to food proteins is still not completely understood, but our previous data strongly suggests a profound contribution of intestinal epithelial stress or damage. This fits with the immunological concept that sensitization requires costimulatory help that can be induced by danger signals, such as arising from tissue damage.

Deoxynivalenol (DON), a mycotoxin that impairs intestinal barrier function by direct effects on intestinal epithelial cells, may act as danger signal. DON produced by *Fusarium species* is among the most frequently detected contaminants in cereal-based foods, resulting in regular human exposure.

Hence, we investigated whether oral exposure of mice to DON mixed with whey proteins resulted in allergic responses to this food protein. Mice exposed to DON plus whey, but not mice exposed to whey only, showed increased whey-specific IgE levels and acute skin responses upon intradermal whey challenge. Analysis of intestinal tissues, isolated 6h after a single oral dose of DON, revealed increased mRNA expression and decreased protein levels of the tight junction molecule claudin-3 and the adherens junction molecule E-cadherin, confirming epithelial damage. We also observed an increase in IL-33 production that was accompanied by enhanced levels of soluble IL-33 receptor ST2 in serum in whey-sensitized mice.

Together, these results demonstrate that DON facilitates allergic sensitization, possibly via the local activation of IL-33 after epithelial cell stress. Our data therefore illustrates the possible contribution of food contaminants, like DON, in allergic sensitization in humans.

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Enzymatic synthesis of glucosides of trichothecene toxins and zearalenone

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ABSTRACT

Fusarium species infest cereal crops and produce harmful metabolites, the most problematic being trichothecene toxins such as deoxynivalenol (DON) and the estrogenic toxin zearalenone (ZEN). Glucosylated *Fusarium* toxins often co-occur with their parent compounds in cereal based food and feed. Of particular importance is DON-3-glucoside, but glucosides of other relevant trichothecenes [nivalenol (NIV), T-2 and HT-2 toxins] and ZEN have also been identified. Currently, there is still limited information available concerning occurrence and toxicological relevance of such masked mycotoxins. Analytical standards are in most cases not commercially available, which limits possibilities to detect and quantify occurrences in cereals. Furthermore, it is crucial to obtain such compounds in sufficient amounts to make toxicological studies possible and to evaluate the role of glucosylation in plant defence. Our group has identified and studied several defence-related plant UDP-glucosyltransferases (UGTs). A rice UGT (OsUGT79) is able to efficiently conjugate DON, NIV and HT-2 toxin. A related UGT from barley (HvUGT13248) in addition can glucosylate T-2 toxin and fusarenon X, but is difficult to express. HvUGT14077, another UGT from barley is highly efficient with ZEN and its phase I metabolites α - and β -zearalenol, yielding the respective 14- and 16-glucosides. We applied these catalysts to synthesize an array of masked mycotoxins including several previously unavailable metabolites. Glucosylated compounds were purified by preparative chromatography and structurally analysed by NMR spectroscopy, showing that enzymatic synthesis exclusively yielded β -D-glucosides. Thus, we are now able to produce the 3-O- β -D-glucosides of DON, NIV, T-2 toxin, HT-2 toxin, fusarenon X, and the 14-O- and 16-O- β -D-glucosides of ZEN and α/β -zearalenol.

Keywords: Masked mycotoxin, trichothecene, zearalenone, Fusarium

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Investigation of multi-mycotoxin occurrence in Nigerian cereals using LC-MS/MS

Cynthia Adaku Chilaka¹; Marthe De Boevre¹; Olusegun Atanda²; Sarah De Saeger¹

ABSTRACT

In recent years, sub-Saharan Africa (SSA) has focused its attention to the contamination of food products with *Aspergillus*-produced mycotoxins, namely aflatoxins thus neglecting other mycotoxins (*Fusarium* toxins) and their subsequent associated hazards. This focus is probably because *Fusarium* mycotoxins are associated to the temperate climates. However, recent trends in climate change seem to have exposed SSA to these toxins. SSA has been reported as a region of higher vulnerability to the impact of global climate change, because of its sole dependence on the weather and climate variables for agricultural production. Cereals are the major food crops affected by *Fusarium* mycotoxins. Information on the occurrence of *Fusarium* mycotoxins in Nigerian cereal crops are limited. This study reports the incidence of *Fusarium* mycotoxins in Nigerian cereal crops, namely: maize, sorghum, and millet. A total of 333 samples comprising of maize (136), sorghum (110), and millet (87) were collected in 2015 from randomly selected markets in Nigeria. Samples were analysed and quantified for *Fusarium* mycotoxins contamination using a multi-mycotoxin liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Overall, 61% of the samples were contaminated with at least one toxin, at the rate of 77%, 44%, and 59% for maize, sorghum, and millet, respectively. Fumonisin were the most prevalent especially in maize at the rate of 65% with mean and median values of 935 µg/kg and 585 µg/kg, respectively. Other mycotoxins detected were diacetoxyscirpenol, deoxynivalenol, zearalenone, and their metabolites, nivalenol, fusarenon-X, and HT-2 toxin. About 38% of the samples were contaminated with more than one toxin. This study suggests that consumption of cereals and cereal-based products may be a source of exposure to *Fusarium* mycotoxins.

Keywords: Cereals, Fusarium mycotoxins, LC-MS/MS, Nigeria, occurrence

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Comprehensive assessment of exposure of Czech population to polycyclic aromatic hydrocarbons

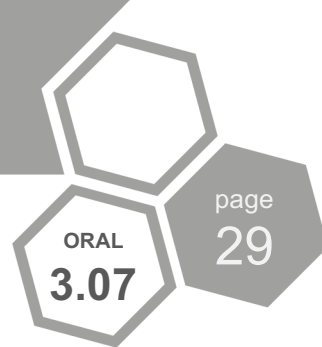
Jana Pulkrabová¹; D. Lankova¹; K. Urbancova¹; A. Svarcova¹; T. Grambicka¹;
Radim Sram²; Jana Hajslová¹

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), produced by incomplete combustion of organic materials, are ubiquitous contaminants present in the environment. We assessed the concentrations of PAHs in samples of (i) air, (ii) one week diet of pregnant women, (iii) human breast milk and (iv) OH-PAHs in urine of both mothers and their newborns living in two regions of the Czech Republic differing in atmospheric contamination by PAHs, specifically the industrial city Karvina and the less industrialized Ceske Budejovice. Samples were collected during two periods August-October 2013 and January-April 2014. The newly optimized and validated analytical procedures for the determination of parent PAHs (n=24) using gas chromatography in combination with tandem mass spectrometry (GC-MS/MS) were applied for their analysis in filters, diet and breast milk samples. In case of hydroxylated metabolites of PAHs (n=11) in urine, a novel sample procedure with ultra-high performance liquid chromatography (UHPLC) hyphenated to MS/MS was used. The results of this unique study focused on a critical assessment of the impact of atmospheric pollution by PAHs on the total body burden of human population resident here, can be summarized as follows:

- The importance to monitor both the dietary in-take together with exposure via inhalation was documented.
- In summer period, the major part of exposure (60–90%) is via diet while in winter more than 60% is via inhalation
- Levels of PAHs in human milk are very low but still significantly contribute to the total body burden of newborns, by about 20–50% of the total exposure depending on the season.
- OH-PAHs were measured as biomarkers in urine, approx. 2x higher median Σ OH-PAHs in urine from winter period in Karvina compared to Ceske Budejovice which is in a good agreement with the data in exposure sources.

Acknowledgments: This research was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195, by the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSM-43760/2015) and by the Czech Science Foundation Project No. 13-13458S.



Highly sensitive analysis of underivatized polar pesticides in food matrices

Euan Ross³; Benjamin Wuyts¹; Dimple Shah²; Eimear McCall³

ABSTRACT

The presence of pesticides in food commodities is a major concern for human health due to their potential toxic nature. Maximum residue levels (MRLs) are in place for many of these compounds, resulting in the need for sensitive detection. The chromatographic analysis of polar pesticides can be challenging since these compounds often lack retention on reversed phase columns. Common alternatives include derivatization and ion chromatography. However, due to time-consuming sample preparation, MS incompatible solvents and the need for specialized equipment and/or reagents, the underivatized LC-MS/MS approach is still preferred. Therefore, we present a direct analysis method for highly polar pesticides, which gives sufficient retention time by the use of a HILIC-type column and provides excellent sensitivity using tandem quadrupole mass spectrometry.

A panel of 8 anionic polar pesticides (AMPA, Chlorate, Ethephon, Fosetyl aluminium, Glufosinate, Glyphosate, Maleic hydrazide, Phosphonic acid) was analyzed in three different food matrices (apple juice, tomato juice, beer). High concentrations of Chlorate, Maleic hydrazide and Phosphonic acid were found in apple and tomato juice. Chlorate and Phosphonic acid were detected in beer. The standard addition method was used to quantify these initial pesticides concentrations, which ranged between 14 and 688 ng/mL. In a next step, the method's performance was critically evaluated by assessing linearity, repeatability and sensitivity. Satisfying linearity was found for all pesticides in a range from 1 to 250 ng/mL in all matrices ($R^2 > 0.995$, residuals $< 20\%$). The robustness of the method was tested using three pre-extraction spiked concentration levels (10, 50, 100 ng/mL). RSD values below 10% were found for all pesticides ($n=9$). Furthermore, the Xevo TQ-XS showed high sensitivity for the pesticides, demonstrated by LLOQ values ($S/N = 10$) below 1 ng/mL in all matrices (except for Ethephon in tomato juice, LLOQ = 2.5 ng/mL).

A separate LC-MS/MS methodology for cationic polar pesticides will also be presented during this talk, outlining method conditions and the analysis of different food matrices.



Climate change – how could it influence risk assessment of food contaminants

Mari Eskola¹

ABSTRACT

European Food Safety Authority (EFSA)¹ carries out risk assessments on food and feed safety at the European level. In the European food safety system, risk assessment is undertaken independently from risk management. As the risk assessor, EFSA produces scientific opinions and advice as the basis of a sound foundation for European policies and legislation. Thus, EFSA supports the European Commission, European Parliament and European Union Member States in their risk management decisions. EFSA's remit is wide and covers food and feed safety, nutrition, animal health and welfare, plant protection and plant health. EFSA's Scientific Panels and Committee have crucial roles in developing its scientific opinions. The experts of the Scientific Panels and Committee come from all over Europe and the world to contribute to the scientific opinions. The EFSA Panel on Contaminants in the food chain (CONTAM Panel)² does risk assessments in the area of chemical contaminants in food and feed, namely process contaminants, environmental contaminants, natural toxicants, mycotoxins and residues of unauthorised substances. Most of the risk assessments were developed upon request from the European Commission.

While EFSA is only now initiating its activities with regard to the climate change, it has already been seen in the some areas of risk assessment on contaminants, such as mycotoxins in food and feed, and marine biotoxins. This presentation gives an overview of the risk assessments on contaminants in food at the European level and how the changing climate could influence these assessments. Some thoughts are also presented on the latest activities of EFSA in the other areas of its risk assessments e.g. on mixtures of contaminants.

References: [1] <http://www.efsa.europa.eu/>
[2] <http://www.efsa.europa.eu/en/panels/contam.htm>

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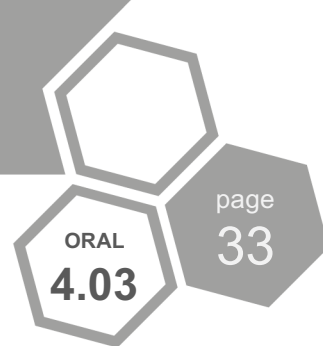
The polar bear in my plate: psychological distance and its association with consumers' perception of climate changeRui Gaspar^{1,2}**ABSTRACT**

Climate change, its associated events and socio-physical consequences, have been posing new demands to citizens, health authorities, and policy makers worldwide. Recently, there have been calls for a better understanding of psychological processes (see e.g. Clayton et al, 2015), for example in how individuals perceive such changes and events, and appraise the demands posed by them. This is a key aspect in order to enable more effective adaptation to those demands (Domingos, Gaspar, Marôco & Beja, 2017). Research in this regard as shown that climate change is appraised as “psychologically distant”, i.e. perceived as something temporally, socially, and geographically distant, and characterized by uncertainty (Spence, Poortinga & Pidgeon, 2012). Despite being psychologically distant for some, climate change and its consequences are physically proximate to all. This seeming contradiction requires the implementation of communication strategies to reduce such psychological distance (see e.g. Jones, Hine & Marks, 2017) in order to capacitate and motivate people to act and implement climate change adaptation strategies.

Within this scope, results from studies on climate change perception in European countries and in Portugal (including adults and children), will be presented. These will focus on psychological processes in general and particularly on the psychological distance component. Implications of such results for climate change communication will be discussed, along with implications for food risk communications particularly considering the sustainable consumption dimension.

Keywords: Climate change perception, climate change adaptation, psychological distance, risk communication

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Aflatoxin exposure through food consumption – are we ready to face the risk associated to climate change?

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ABSTRACT

Climate change (CC) has been indicated as a driver for food safety issues worldwide, mainly due to the impact on the occurrence of food safety hazards at various stages of food chain. Mycotoxins, natural contaminants produced by fungi, are mentioned to be one of the most important food safety hazards affected by CC. Aflatoxins, which have the highest acute and chronic toxicity of all mycotoxins, assumes particular importance within this context. A recent study predicted aflatoxin contamination in maize and wheat crops in Europe within the next 100 years¹. The authors concluded that aflatoxin B₁ (AFB₁) is predicted to become a food safety issue in Europe, especially in the +2 °C scenario, the most probable scenario of CC expected for the next years. In Europe, previous reported studies estimated mean dietary exposures to aflatoxins for the general population from all food sources ranging from 0.93 ng kg bw⁻¹ day⁻¹ to 2.4 ng kg bw⁻¹ day⁻¹². In Portugal, an estimation of a probable daily exposure to aflatoxins of 0.501 ng kg bw⁻¹ day⁻¹ by the Portuguese population was reported³. In addition, other authors also estimated aflatoxin exposure of Portuguese children (1-3 years) and reported a potential health concern associated to the exposure, wherein AFB₁ was the main contributor⁴.

Considering the potential risks associated to Portuguese exposure to aflatoxins through diet, and the potential influence of CC on the temperature, humidity, precipitation and consequently on the mycotoxin contamination of food products, this presentation will discuss the CC impact on public health. To face this objective, burden of disease (BoD) associated to aflatoxin exposure of the Portuguese population will be estimated, for the first time, using a model of three components: an exposure, health-outcome and disability adjusted life years (DALY) modules⁵. BoD of different scenarios will be discussed in light of the current knowledge about recent estimates of CC impact in Europe.

Acknowledgments: This research was performed under National Institute of Health Dr. Ricardo Jorge (Projecto Incentivo MICOTOXINAS) and CESAM by the Portuguese Foundation for Science and Technology (FCT) (UID/AMB/50017/2013), through national funds, and the co-funding by the FEDER (POCI-01-0145-FEDER-00763), within the PT2020 Partnership Agreement and Compete 2020.

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Interacting climate-related environmental factors: impacts on colonisation and mycotoxin contamination of food commodities

Angel Medina¹; Naresh Magan¹

ABSTRACT

Lately, there has been a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have now studied for the first time the effect that three way interactions between water availability, temperature and elevated CO₂ have on different mycotoxin producing fungal species. Among them in this presentation we will present the main results obtained for *Aspergillus flavus*.

For this important species we have studied effects on (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B₁ production on both conducive media and stored maize. Fungal growth, AFB₁ production and expression of the all genes in the aflatoxin gene cluster by RNAseq were studied. The results in culture media showed growth was relatively unaffected. In contrast, the three-way interacting conditions had a profound effect on aflatoxin B₁ production both in media and maize grains. Under slightly elevated CO₂ conditions there was a stimulation of aflatoxin B₁ production.

In stored maize grain, differential expression of several genes in the aflatoxin gene cluster where found in relation with these interacting factors. Aflatoxin B₁ production increased under elevated CO₂ conditions at both temperatures and a_w tested.

These are the first studies to examine these three-way interacting climatic factors on growth and mycotoxin production in different fungal species including *A. flavus*.

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Effects of storage, processing and digestion on the cyanotoxins microcystin-LR and cylindrospermopsin: the importance in its integration in the human health risk assessment

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ABSTRACT

The occurrence and proliferation of toxic cyanobacterial blooms are an emergent environmental concern. Microcystin-LR (MC-LR) is the most documented cyanotoxin, while cylindrospermopsin (CYN) has been recognized of increased concern due to the invasive nature of its main producer. Previous studies have shown that bivalves can accumulate high levels of these cyanotoxins. MC-LR and CYN are stable at a wide range of temperatures and pHs, thus the knowledge of the influence of storage and processing, as well as human digestion on its concentration in food is required to achieve a more accurate health risk assessment. The aim of this study was to assess the influence of storage, processing and human digestion on the concentration of MC-LR and CYN in edible bivalves. Clams fed microcystin-producing *M. aeruginosa* and mussels fed cylindrospermopsin-producing *C. raciborskii*, were subjected to storage and processing techniques over different periods and then analyzed by LC-MS/MS. Bioaccessibility of MC-LR and CYN were assessed by *in vitro* digestion. Overall, cooking for short periods of time resulted in a significantly higher concentration of free MC-LR in clams: 57.5-59% and 163.4-213.4% in microwave and boiling treatment, respectively. Mussels stored frozen allowed a significantly higher recovery of CYN (52.5-57.7%). CYN was found in the cooking water, suggesting that heat processing can be used to reduce the availability of CYN in this food item. The bioaccessibility of MC-LR was reduced to 83%, potentially because of MC-LR degradation by pancreatic enzymes. The *in vitro* digestion with salivary and gastrointestinal juices considerably decreased the CYN availability in mussels. Our results suggest that risk assessment based on MC-LR and CYN concentration in raw products might not be representative of true human exposure, once the techniques commonly used for their preservation and processing as well as bioaccessibility can strongly change the potential toxicological risks.

Keywords: Bioaccessibility, bivalves, cylindrospermopsin, Microcystin-LR, risk assessment

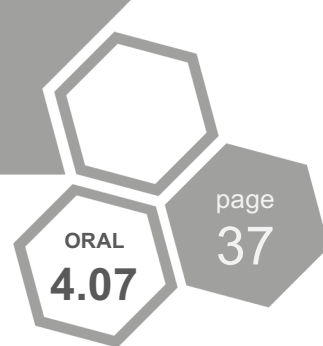
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Management and mitigation strategies for mycotoxins: the biological solutionsLuís Abrunhosa¹**ABSTRACT**

Mycotoxins are toxic secondary metabolites produced by fungi that are found worldwide in many food products. They are a diverse group of chemical compounds that possess a multiplicity of toxic properties and affect human and farm animal health. Additionally, they cause significant economic losses. The problem of mycotoxins results mainly from fungal growth on crop cultures at the pre- or post-harvest stages. Implicated fungi belong mostly to *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* genera. For these reasons, mycotoxins presence in foods should be monitored, and their levels reduced as low as technologically possible. In recent years, several biological solutions have been studied and developed to mitigate the adverse effects of mycotoxins. These strategies can be divided into three main type of action: i) Biocontrol, in this case, defined as the control of mycotoxigenic fungal growth by other microorganisms; ii) Adsorption, which refers to the use of microorganism cells to bind mycotoxins; and iii) Biotransformation, when microorganisms or pure enzymes are used to degrade or transform mycotoxins into non-toxic compounds. This presentation will revise some of the main achievements done in this field in the last years and present the work done at the Centre of Biological Engineering related to this thematic. Presented topics will include: i) The inhibition of *Aspergillus flavus* and *Penicillium nordicum* growth, as the production of aflatoxins and ochratoxin A by lactic acid bacteria and their organic acids; ii) The adsorption of mycotoxins by microorganisms isolated from Kefir grains; and iii) The biodegradation of ochratoxin A by *Pediococcus parvulus*.

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Keywords: Mycotoxins, fungi, biocontrol, adsorption, biotransformation



Assessment of potentially toxic elements in macroalgae grown in an Integrated Multi Trophic Aquaculture (IMTA) system

Liliana Duarte ¹; Bruno Henriques ^{2,3}; Eduarda Pereira ²; Helena Abreu ⁴

ABSTRACT

Macroalgae consumption by humans has been increasing in the last years, which is related with their nutritional value with health benefits. Following the need to increase their production in a sustainable way, the Integrated Multi Trophic Aquaculture (IMTA) that involves more than one trophic level emerged as a promising alternative to conventional aquaculture. Quality assurance and food safety are crucial, and that is why the present study is so important, especially when macroalgae are known to be high bioaccumulators of potentially toxic elements (PTEs). The objective of this work was to monitor the concentration of Cd, Al, Pb, and Hg in *Ulva rigida* grown in a commercial scale land based IMTA system in ALGApplus, Aveiro, Portugal. Possible correlations between PTEs concentration and cultivation conditions (flow rate and stock density) and seasonality (summer to winter) were also assessed. Samples were collected from 12 different tanks, corresponding to three replicates per condition, during the period of July 2016 to March 2017. Seaweeds remained in the tanks for 15 days and grew by vegetative propagation. The contents of Cd, Pb, Al and Hg in macroalgae biomass were determined by ICP-MS, after microwave-assisted acid digestion, while Hg concentration was directly determined by thermal decomposition and atomic absorption spectrometry on a LECO® AMA-254. Results showed that seasonal variability is not significant ($p > 0.05$) to the accumulation of PTEs by *U. rigida*. However statistically differences among conditions and between replicates were found ($p < 0.05$), which highlights the importance of manipulating culture parameters as a tool to prevent potential consumer risks. All the PTEs concentrations were below the values imposed by the legislation, which proves the safety of this IMTA system.

Keywords: Macroalgae, bioaccumulation, cultive conditions, food industry, land-based IMTA system, potentially toxic elements

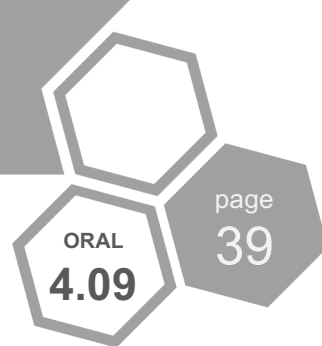
Biotechnological potential of a laccase mediator system for AFM₁ degradation

Martina Loi¹; Francesca Fanelli¹; Laura Quintieri¹; Vania C. Liuzzi¹;
Miriam Haidukovski¹; Antonio F. Logrieco¹; Giuseppina Mulè¹

ABSTRACT

Climate change is one of the major factors affecting the ecology of mycotoxin producing fungi. In the next years, mycotoxin contamination is expected to dramatically increase. In this scenario, prevention strategies hardly counteract the evolving mycotoxin contamination pattern and alternative methods for mycotoxin control are needed to ensure food safety within the whole supply chain. Aflatoxin M₁ (AFM₁) is a common contaminant of milk and dairy products as carry over of aflatoxin B₁. It represents a great health concern worldwide since it is classified in group 2B by the International Agency on Research on Cancer, thus possible carcinogenic to humans. In this study, the application of a laccase mediator system (LMS) was studied for AFM₁ degradation *in vitro* and in skim UHT milk. Laccase alone as well as with five different natural and artificial mediators was tested. An *in vitro* screening was performed in acetate buffer 1mM pH 6.5 for 72h at 25°C. Time course degradation was then evaluated *in vitro* and in skim UHT milk. Laccase alone was not able to degrade AFM₁, while the addition of any of the tested mediator lead to a significant reduction (68%) or complete removal (100% degradation) of the toxin within 72h. In buffer solution AFM₁ was halved within 1h of incubation and reduced by 90% after 24h with a logarithmic fit. The same trend was registered in milk, though the rate of degradation was slower in the first hours of assay. LMS crosslinking activity on milk proteins was also investigated. Preliminary data showed that caseins and lactoglobulins are involved in oligomer formation, with important potential implication in the rheology and allergenicity of milk based products. The application of laccase enzymes in combination with natural redox mediators is a promising strategy to reduce AFM₁ contamination. The green feature of LMS and the effectiveness in real food matrix open new perspectives for AFM₁ control and to ensure the safety of milk and dairy products.

Keywords: Aflatoxin M₁, laccase, redox mediator, degradation



Food safety in wine: the application of oenological products to reduce aflatoxins from contaminated white and red wines

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ABSTRACT

Aflatoxins (AFs) are secondary metabolites produced by certain moulds of the genus *Aspergillus*. These mycotoxins are difuranocoumarin derivatives produced *via* the polyketide pathway mainly by *A. flavus* and *A. parasiticus*. There are about 20 types of AFs, being AFB₁ the most toxic and prevalent of them [1]. AFB₁ is the most potent carcinogen known among natural toxins being classified as a Group 1 compound (carcinogenic to humans) by the International Agency for Research on Cancer [2]. AFs and the fungi that produce them can also contaminate grapes used for wine production. Recently, AFs were detected in some wines [3-5]. Despite its toxicity, the maximum permissible content in grape juice and wine is not regulated, contrary to what happens with ochratoxin A (<2.0 µg/kg), therefore it is important to prevent and control its occurrence in wines [6-8]. This work aims to study the effectiveness of ten oenological products with different characteristics on the reduction of artificially contaminated white and red wine with AFB₁ and AFB₂ at a final concentration of 10 µg/L. For AFB₁ the oenological product that showed to be the most effective was calcium bentonite, with 100% removal in both wines, followed by potassium caseinate with 93% and 72% removal in white and red wine, respectively. Regarding AFB₂, the most effective oenological products was again calcium bentonite with 100% and 81% removal in white and red wine, respectively followed by activated carbon with 86% removal in white wine and potassium caseinate with 62% removal in red wine. In addition, the impact of these oenological products on the wine physicochemical characteristics was also evaluated. The results obtained can provide useful information for the wine sector, for the choice of the most appropriate oenological product to reduce/remove aflatoxins from wine, reducing their toxicological potential and the risk for consumers, without losing the sensory quality and physicochemical properties of the wine.

Keywords: Aflatoxins, oenological products, wine, color, phenolic compounds

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POSTER ABSTRACTS

**Study on the possibility of aflatoxin M1 milk contamination from ingestion of contaminated feed by dairy cattle**

Ana Gonçalves¹; Armando Venâncio¹

ABSTRACT

Aflatoxin contamination is a major issue in food and feed safety fields. In fact, the presence of aflatoxin B₁ (AFB₁) has a wider impact since its presence in feed can lead to contaminated milk, because the toxin is metabolised in aflatoxin M₁ (AFM₁) by dairy cattle which feed contains the mycotoxin. In the EU, the maximum legal level for AFM₁ in milk is 0.05 µg/kg, while for AFB₁ it is 5 and 20 µg/kg, for compound feed for dairy cattle and feed raw materials, respectively. Based on relevant data, calculations on the expected level of AFM₁ in milk were made, as well as on the AFB₁ content in feeds that assures compliance of milk with current legislation. A feed consumption of 18.5 to 26 kg and toxin carry-overs of 1, 2 and 6.2 % were considered. Regarding the predicted AFM₁ levels in milk, the highest value was 0.28 µg/kg, for the worst tested case - highest carry-over and all feed at the highest allowed level for AFB₁. This value is over 5 times higher than the EU limit for AFM₁ in milk. Considering the assessment of the necessary level of AFB₁ in feed to ensure a compliant milk, findings suggested that the mean concentration in dairy cattle feeds should be, in some scenarios, lower than the regulated limits (range: 1.55 to 6.76 µg/kg). The results show the possibility of compliant feed originating milk contaminations above the legal limit. Even so, because it is not expected that dairy cattle's entire consumption of feed has the maximum concentration level of AFB₁, AFM₁ levels in milk would be lower than the obtained. In fact, it is known that the incidence of milk samples with AFM₁ above 0.05 µg/kg is around 0.06 % (EFSA, 2004). The relevance of this subject is even higher since according to an EFSA report (2012) the expected climate changes - higher temperatures and drought - will lead to an increase on mycotoxin production and have a negative impact on this matter.

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Potential effects of environmental conditions on the efficiency of prothioconazole plus tebuconazole to control *Fusarium sporotrichioides* growth and T-2 and HT-2 biosynthesis in oats

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Rufino Mateo-Castro²; Misericordia Jiménez¹; Eva M. Mateo¹

ABSTRACT

Oats (*Avena sativa* L.) is one of the most important crops worldwide. The health-beneficial properties of oats have led to an increase in the consumption of oats and oat-based food products in recent years. In previous researches, *F. sporotrichioides* was the predominant species detected in Spanish oat samples. We also found that a very high percentage of the isolates of this species were T-2 and HT-2 toxin producers. The aim of this study was to assess the effects of environmental conditions on the efficiency of Prosaro®, a commercial azole formulation, to control fungal growth and T-2 and HT-2 biosynthesis by *F. sporotrichioides* isolated from Spanish oats. Oat grains, previously analyzed to ensure they had undetectable levels of T-2 and HT-2 toxins, were placed in Erlenmeyer flasks and autoclaved for 20 min at 121 °C were supplemented with 0.3, 0.6, 0.9 and 1.2 µg/g of Prosaro® [Prothioconazole 12.5% p/v (125 g/L) + Tebuconazole 12.5% p/v (125 g/L)] (Bayer CropScience, Spain) were used. Ecological conditions were: i) a_w , 0.95, 0.97 and 0.99, ii) temperature, 20, 22, 24, 26, 28 and 30 °C. Linear regression of colony radius (mm) against time (days) was used to determine growth rates (GR). Toxins in oat cultures were determined by LC-DAD. ANOVA revealed that a_w , temperature and fungicide doses had significant effects on the *F. sporotrichioides* GR and on T-2 and HT-2 biosynthesis in oat grains. GR followed the order $0.99 > 0.97 > 0.95$ a_w ($p < 0.05$). However, toxin production increased in the order $0.97 > 0.99 > 0.95$ a_w ($p < 0.005$). GR and T-2 and HT-2 biosynthesis decreased with increasing fungicide doses, regardless of the a_w and temperature values.

Acknowledgements: The authors acknowledge financial support from the ERDF and Ministry of Economy and Competitiveness (MINECO) (Spanish Government) (Project AGL2014-53928-C2-1-R and predoctoral contract, BES-2015-071242) and Generalitat Valenciana (postdoctoral contract, APOSTD/2016/102).

Keywords: *Fusarium sporotrichioides*, azole fungicides, tebuconazole, prothioconazole, oats, T-2 and HT-2 toxins, environmental conditions

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Influence of temperature and a_w growth and OTA biosynthesis by *Aspergillus carbonarius* and *Aspergillus ochraceus* in synthetic culture coffee base

Gislaine Oliveira ¹; Fabiana Passamani ¹; Sara Chalfoun ²; Suzana Evangelista ¹; Luís Batista ¹; Maria Cardoso ¹; Wilder Santiago ¹

ABSTRACT

The OTA development and biosynthesis by toxigenic fungi depend on climatic factors, the temperature and a_w those who play the most important role. These factors are relate to microbiological growth, and have the ability to influence the expression of genes involved in the biosynthesis of mycotoxins. This study was conducted to evaluate the *in vitro* influence of temperature and a_w growth and OTA biosynthesis by *Aspergillus carbonarius* and *Aspergillus ochraceus* in synthetic culture coffee base. To evaluate the effect of abiotic factors in the growth of these fungi and in the OTA biosynthesis *in vitro* conditions, it was used as a tool, a central composite design. The isolates producers of OTA were cultivated on synthetic culture coffee base with different water activity (0.99, 0.98, 0.95, 0.92 and 0.91) and incubated at different temperatures (17 ° C 20 ° C, 27.5 ° C, 35 ° C and 38 ° C). The isolates of *A. carbonarius* CCDCA10288 and CCDCA10293 showed the largest increase in a_w ranges from 0.935 to 0.965, and temperatures between 25 ° C to 32 ° C. Similarly, the optimum growth conditions for the isolates of *A. ochraceus* CCDCA10211 and CCDCA10212 occurred in the a_w intervals between 0.940 to 0.990, and temperatures between 21 ° C to 30 ° C. The biggest amount of OTA produced by *A. ochraceus* CCDCA10288 and CCDCA10293 (19.7 and 15.7 µg/g, respectively) was obtained at a_w 0.99 and temperature of 15 ° C to 25 ° C. There was a trend for increased OTA production by *A. ochraceus* CCDCA10211 and CCDCA10212 (8.9-7,9 µg/ g, respectively) in a_w of 0.98 to 0.99, and temperatures between 25 ° C to 35 ° C and 22 ° C to 32 ° C, respectively. The temperature and a_w affect on the production of OTA is understood more easily when occur the information grouping and the development of models that simulate weather scenarios, providing adaptation strategies.

Keywords: Food safety, mycotoxins, *Aspergillus*

Effect of abiotic factors on growth and production of ochratoxin A by *Aspergillus carbonarius* and *Aspergillus ochraceus* isolated from Brazilian

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Sara M. C. Souza ²; Luís Roberto Batista ¹

ABSTRACT

This study was carried out with the objective of evaluating *in vitro* the influence of temperature and a_w on the growth and biosynthesis of Ochratoxin A (OTA) by *Aspergillus carbonarius* and *Aspergillus ochraceus* in a green coffee-based medium. These isolates were obtained from green coffee in the southern region of Minas Gerais (Brazil) and previously evaluated in relation to the production capacity of OTA. The effect of these abiotic factors was evaluated using a Central Composite Rotatable Design with a_w ranging from 0.91 to 0.99 and temperatures ranging from 17 °C to 38 °C. Isolates of *A. carbonarius* CCDCA10288 and CCDCA10293 showed optimal growth conditions in a_w intervals from 0.935 to 0.965 and temperature between 25 °C and 32 °C and isolates of *A. ochraceus* CCDCA10211 and CCDCA10212 in a_w intervals from 0.940 to 0.990 and temperature between 21°C to 30°C. The highest production of OTA by *A. carbonarius* CCDCA10288 and CCDCA10293 (19.7 and 15.7 µg/g respectively) was in the following conditions a_w of 0.99 and temperature between 20 °C and 25 °C and *A. ochraceus* CCDCA10211 and CCDCA10212 (8.9 and 7.9 µg / g respectively) in a_w between 0.98 and 0.99 and temperature between 25 °C and 30 °C and 22 °C and 32 °C respectively. Using the models obtained and the estimation of maximum and minimum temperature of the south of Minas Gerais, it was possible to verify that due to the increase in minimum temperatures the condition becomes favorable for ochratoxin A production in the months of December to March. However, in this period the coffee fruits is mostly in the immature stage, not being favorable substrate to the development of the ochratoxigenic fungi. Therefore, the increase in annual temperatures will not affect the sanitary quality of the coffee produced in this region by the presence of OTA.

Financial support: FAPEMIG, CNPq.

Keywords: Mycotoxins, coffee, ochratoxigenic fungi, temperature



Essential oils as potential agents to control *Aspergillus flavus* and *A. parasiticus* and aflatoxin production in food

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ABSTRACT

Climate change can influence the life cycle of microorganisms including toxigenic fungi and mycotoxin production in crops. In this line, *Aspergillus flavus* and *A. parasiticus*, the major aflatoxin-producing species in crops, are and may become strong competitors in new scenarios associated to climate change. An increase in mycotoxin levels in food due to reduced use of fungicides in crop production has been reported. Thus, alternative treatments are being studied. Essential oils (EOs) from plant (tissues or seeds) are a promising strategy. EOs are considered GRAS (“Generally Recognized As Safe”) by the FDA and they have the potential advantage of being bioactive in their vapor phase, a characteristic that makes them attractive as possible fumigants of stored products. The objectives of this study were to assess the efficacy of essential oils: oregano, cinnamon and their major active constituents, carvacrol and cinnamaldehyde, respectively on: i) control of growth of *A. flavus* and *A. parasiticus* isolates under different environmental conditions and ii) control of aflatoxin production by these two species. Effective dose for 50 and 90% (ED₅₀ and ED₉₀) growth inhibition and toxin production were determined. Growth rate (GR) of *A. flavus* and *A. parasiticus*, was higher at 37 °C than at 25 °C and at 0.99 than at 0.95 a_w. GR generally decreased with increasing EOs doses regardless of a_w, temperature, fungal species and type of EO. However, different response profiles were observed depending on the EO type. The effectiveness to control fungal growth and aflatoxin production was: cinnamaldehyde > carvacrol > oregano and cinnamon.

Acknowledgements: The authors acknowledge financial support from the ERDF and MINECO (Spanish Government) (Project AGL2014-53928-C2-1-R and predoctoral contract, BES-2015-071242) and Generalitat Valenciana (postdoctoral contract, APOSTD/2016/102).

Keywords: Essential oils, *Aspergillus flavus*, *Aspergillus parasiticus*, aflatoxins

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Mycotoxycological and nutritional aspects of organic spelt and common wheat grain and its products

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Danutė Jablonskytė-Raščė³

ABSTRACT

The study was done at the Lithuanian Research Centre for Agriculture and Forestry (LAMMC) in 2011-2013. Common winter wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.) grain was grown under organic production conditions on *Endocalcari Endohypogleyic Cambisol*. The study aimed to quantify and compare mycotoxins (deoxynivalenol, zearalenone and T-2/HT-2 toxin), nutritional potential and technological properties in the grain (and glumes) of spelt and grain of common wheat as influenced by changing climate conditions. Spelt grain was found to be less contaminated by mycotoxins than common wheat. Spelt glumes were more contaminated with toxins than grain. The highest mycotoxins concentrations in wheat grain and spelt glumes were identified in 2011, which was warmer and wetter compared with the other experimental years and the long-term average. In 2011, the concentrations of T-2/HT-2 toxin in spelt glumes exceeded the safe level and were 5.4 times higher in common wheat grain and 7.4 times higher in spelt glumes than in spelt grain. Spelt glumes were noted to perform a protective function against mycotoxycological contamination. Spelt grain had higher protein, fibre and mineral (especially phosphorus) content compared with common wheat. The grain of spelt was softer and its flour yield was higher. Farinograph analysis revealed that spelt flour absorbed less water; dough formation and stability took more time compared with that of common wheat. Organic spelt grain was characterised by higher nutritional value and higher crop resistance to environmental stress in the climate change conditions, which resulted in lower mycotoxin contamination compared with the grain of common wheat. Therefore, spelt grain is not only healthy but also safe food product.

Acknowledgements: This study was supported by the long-term research programs 'Harmful Organisms in Agro and Forest Ecosystems' and 'Biopotential and Quality of Plants for Multi-functional Use' implemented by LAMMC.

Keywords: Triticum spelta, Triticum aestivum, organic grain, mycotoxins, chemical composition, technological properties

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Sterigmatocystin in foodstuffs: higher concern due to climate changes?

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ABSTRACT

The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the cereals produced in the world are contaminated with mycotoxins. There are many factors involved in mycotoxins production by fungi but the climate is the most important. The climate of some countries will probably become warmer reaching temperatures of 33 °C, which is, for instance, a temperature very close to the optimal for several mycotoxins production. This is the case of sterigmatocystin (STC), produced mainly by *Aspergillus versicolor*. Although its toxicity is lower than AFB₁, STC is classified as being carcinogenic classified by IARC as group 2B, with immunomodulatory activity, mutagenicity in mammalian cells after metabolic activation, inhibition of the cell cycle and mitosis, and an increase in formation of reactive oxygen species and lipid peroxidation *in vivo*. This review intended to collect information present in scientific papers about STC presence in foodstuffs and an exhaustive search was made for papers from January 2010 available on online scientific databases. Papers reported the occurrence of STC in basic daily foods at low levels such as wheat, rye, corn, barley and by-products, bread, soy, groundnuts, rice, cocoa beans, vegetables and pistachio, and at high levels in red pepper, caraway, cumine (18-23 µg.kg⁻¹) and coffee beans (12 000 µg.kg⁻¹). Although in most of the foods the levels found are low, there is still a concern since it can lead to a chronic exposure that, in some countries, can result in higher exposure to the presence in several foodstuffs. So, further studies are needed in order to assess exposure considering different diet regimes. Additionally, research projects should be dedicated to understand if the contamination by STC in different crops and products is expected to increase due to climate change. This will allow to recognize the most suitable measures to avoid contamination and prevent consumers exposure.

Keywords: Sterigmatocystin, food contamination, climate change



Exposure assessment of infants to aflatoxin M₁ in breast milk and maternal social-demographical and food consumption determinants

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Humberto Rocha ¹; Celeste Lino ²; Angelina Pena ²

ABSTRACT

Aflatoxin M₁ (AFM₁; group 2B IARC) which can be transmitted to newborns via breast milk, is a hydrolyzed metabolite of aflatoxin B₁ (AFB₁) that is ingested along with contaminated food. The occurrence of AFM₁ in maternal milk and the degree of exposure of infants to this toxin were studied. The correlation between the concentration of AFM₁ and basic socio-demographic factors and the consumption of certain categories of food was also aimed. Thus 30 milk samples from nursing mothers were collected (2016), and analyzed by ELISA, in order to determine the presence of AFM₁. Thirteen samples (43.3%) contained levels of AFM₁ above the detection limit (5ng/L), ranging between 5.1 and 10.2ng/L (7.12±1,89ng/L). Statistical analysis showed a moderated correlation between the maternal consumption of dry fruits (r=0.48) and milk (r=0.4) and the concentrations of AFM₁ found in the samples. No other studied determinants, whether socio-demographic (age, weight, height, number of children, lactation phase, education, professional activity, residence, characteristics of breastfeeding, infants' weight) or dietary (frequency of food consumption) showed a significant statistical influence. AFM₁ estimated daily intake (EDI) was higher for younger babies (1.06ng/kg b.w.; <7kg) as compared with the older ones (≥7kg; 0.8ng/kg b.w.), which can be explained by the higher consumption versus weight. The hazard index for both groups (<7kg b.w.: 5.3; ≥7kg; 4.0) were far greater than 1.0, which is the value that indicate risk for consumers. Results suggest the need to reinforce AFB₁ surveillance in food, particularly dry fruits and milk, as a protective measure, not only for adults, but ultimately for nursing infants exposed by lactation to AFM₁. Although AFM₁ presents lower carcinogenic potency, it is noteworthy that when compared with adults, infants feature a lower capacity of carcinogen biotransformation, a fairly restricted diet and a higher consumption in relation to body weight.

Keywords: Aflatoxin M₁, breast milk, newborns

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Ecophysiology of *Penicillium expansum* and patulin production in synthetic and olive-based mediaHamdi ¹; Jorge Sá-Morais ¹; HEND Bejaoui ²; Paula Rodrigues ¹**ABSTRACT**

The olive and its derivatives, in particular olive oil, represent one of the most significant agricultural products in the Mediterranean basin. Storage under inadequate conditions poses serious problems concerning fungal contamination, with consequent defects and potential mycotoxin production in olives and olive oils. *Penicillium expansum* represents one of the most significant postharvest pathogens in several fruits, including olives. Not only it causes blue mold but also is one of the most relevant patulin (PAT) producing species of the genus *Penicillium*. The aim of this research was to evaluate the ecophysiological conditions governing growth and PAT production by *P. expansum* strains previously isolated from Tunisian olives. For this purpose, four *P. expansum* isolates were tested in a synthetic medium (Czapek Yeast Autolysate, CYA) and in olive-based medium (OM) for their ability to grow and produce PAT under different temperatures (4 °C, 15 °C and 25 °C) for 10 and 20 days. The mycotoxin was analysed by HPLC-UV. Results showed that all isolates were able to grow on tested media at different temperatures. Different PAT production profiles were found, showing that at 25 °C *P. expansum* isolates were able to produce PAT on CYA and OM medium. At 15 °C the production of PAT was only detected on CYA medium, while no PAT production was detected at 4 °C for the two media.

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Keywords: Mycotoxins, storage conditions

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Aflatoxin M₁ in milk from sheep herds

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Humberto Rocha¹; Angelina Pena³; Celeste Lino³

ABSTRACT

Ruminants are considered less susceptible to mycotoxins than monogastrics, because of the rumen microbiota (Gallo *et al.*, 2015). Nonetheless, as dairy species, the carry-over of mycotoxins to milk may represent a source of exposure to consumers. From all the known mycotoxins, aflatoxin B₁ (AFB₁), represents the bigger concern, namely for the carcinogenic toxic effects that its exposure embodies (IARC 1). The main AFB₁ metabolite (aflatoxin M₁; AFM₁; IARC 2B) can be used as a biomarker of exposure. Bulk-tank milk samples (24) from sheep herds were collected at the middle of lactation cycle in center of Portugal (February 2017) and surveyed for AFM₁ occurrence using a competitive ELISA (I'screen, TECNA). Eleven samples (45.8%) were found positive (7.93±2.60ng/L), although none surpassed the EU Maximum level (50ng/L; 1881/2006/EC), as the maximum detected value was 4-times lower (12.55ng/L). However detected levels were significant considering that sheep herds are fed silage for very limited times compared with cows (feed-regime is almost entirely pasture-based) and that analyzed samples were from bulk-tank, and so a dilution effect could occur. The calculation of extrapolated values of AFB₁ concentration in feeds from back calculation of the values of AFM₁ obtained from analysis of milk samples (Battacone *et al.*, 2005) resulted in c.a. 2µg/kg (EU ML 5µg/kg for dairy animals; 2002/32/EC). The determined AFM₁ incidence values were lower than 100%, as reported in Jordan (Omar, 2016) and Croatia (Bilandžić *et al.*, 2014), but higher than in Italy (4.6%; Virdis *et al.*, 2014) and Iran (31%; Rahimi *et al.*, 2012). Although scarce, the studies surveying sheep milk for AFM₁ occurrence are significant because: almost all the produced milk is destined to cheese manufacture; AFM₁ concentration in cheese is estimated to be 2 times higher than in milk (AFM₁ associates with milk proteins); sheep feature a higher concentration of proteins in milk than cows (Battacone *et al.*, 2005).

Keywords: Aflatoxin M₁, milk, sheep

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Patulin bioaccessibility in apple-based juices – characterization of degradation during human digestion

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ABSTRACT

Food products could be contaminated in several ways. Chemical food contaminants as mycotoxins, which are secondary metabolites of fungi, can constitute a serious health problem considering their potential toxic effects. Warm temperatures and water activity are crucial for fungi development and mycotoxin production. Climate change may turn traditional temperate regions like Europe more liable to mycotoxins. Patulin (PAT), a mycotoxin that can promote health problems, is traditionally detected in apples and apple-based foods, including those usually consumed by young children. Since the most frequent route for chemical food contaminants is ingestion, the impact of the digestion process on the availability of these compounds must be well characterized. The present study aimed to evaluate the impact of the digestion process on PAT bioaccessibility, using artificially contaminated apple-based juices (minimum 50% apple content). To attain this objective, human digestion was simulated *in vitro* (according to Minekus et al., 2014) and PAT bioaccessibility was characterized along the three different phases, Oral (O), Gastric (G) and Intestinal (I). PAT identification and quantification were performed according to Barreira et al (2010). At intestinal phase, clear juices showed lower PAT mean bioaccessibility values (32 ± 10.0 %) than cloudy (36 ± 10.2 %). The results also showed that the majority of PAT degradation mainly occur in intestinal phase (mean bioaccessibility: clear O 90%; G 91%; I 32%; cloudy O 72%; G 73%; I 36%). These preliminary results are the first ones describing PAT bioaccessibility along the digestion process, and highlight the need to perform further studies considering different fruit-based products potentially contaminated with PAT. In a risk assessment perspective, these results contribute to a more accurate risk assessment, taking into account the amount of contaminant that is available to be absorbed in the intestine.

Keywords: Patulin, bioaccessibility, mycotoxins, in vitro digestion, children

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Mycotoxins as emerging risks: from dietary exposure to urinary biomarkers

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ABSTRACT

Daily life human exposure to several chemical contaminants is a known reality and ingestion of food products constitutes one of the main routes of this exposure. Mycotoxins, secondary metabolites of fungi, contaminate food and assume particular importance in Public Health, regarding their potential toxic effects. Considering that mycotoxins occurrence in foods has been frequently reported, in diversified foodstuffs, at low concentrations, it is expected a chronic exposure to mycotoxins in the Portuguese population. Biomonitoring is a relevant key tool to accurately characterize this exposure. In addition, as health-based guidance values are established only for food intake, it is of the utmost importance to find a correlation between urinary biomarkers and food intake. Previous studies had reported associations between food intake and mycotoxins related urinary biomarkers, particularly for DON, OTA and FB₁. It is intended, for the first time in Portugal, to estimate the exposure of the population to multiple mycotoxins through the determination of urinary biomarkers and to develop a model of association between biomarkers concentrations and food consumption. An epidemiological, observational and cross-sectional study will be developed, with the analysis of 24-hour urine samples at individual level, in a sub-sample of participants in the recent National Food and Physical Activity Survey. Expected results will contribute: i) to estimate the Portuguese population exposure to mycotoxins, allowing to identify groups of the population more exposed and consequently more vulnerable, and ii) to design intervention strategies at the health promotion level leading to the empowerment of the population to select an adequate diet. Furthermore, and considering the potential consequences of climate change on mycotoxins occurrence in food, this study will allow to anticipate the potential risk associated to mycotoxins exposure in a climate change scenario.

Keywords: Mycotoxins, biomarkers, dietary exposure

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Fungal burden of bread raw material and settled dust from Portuguese bakeriesCarla Viegas ¹; Ana Crespo Batista ²; Liliana Aranha Caetano ^{2,3}; Susana Viegas ¹**ABSTRACT**

The Food and Agriculture Organization estimated that approximately 25% of the cereals produced in the world are contaminated with mycotoxins. Crops protection includes the use of fungicides such as triazoles, also used in clinical settings. In this study we aimed at assessing fungal contamination and resistant species of bread raw material and also settled dust from 5 Portuguese bakeries. Twenty six samples of bread raw material and one settled dust sample from each bakery were assessed. 4.4 g of raw material/settled dust was weighted and added 40 ml of distilled water for extraction (20' at 200 rpm). 150 uL of this suspension was spread onto malt extract agar (2%) with chloramphenicol (0.05 g/L) (MEA), in dichloran glycerol (DG18) and onto screening media to detect azole-resistant fungal isolates. None of the settled dust analyzed presented fungal growth. However, in two bakeries fungal growth was isolated in 1 flour sample (one with *Aspergillus* section *Circumdati*, another with *A.* section *Versicolores*). In a third bakery, 3 different flour samples showed fungal growth (2 with *A.* section *Versicolores*, another with *A.* section *Versicolores*, *Mucor* sp. and *Penicillium* sp.). In a fourth bakery, fungal isolates were identified in 4 samples (1 with *Penicillium* sp., 2 with *A.* section *Candidi*, another with *Syncephalastrum racemosum*). Additionally, the growth of resistant fungi in 1 flour sample was observed in 2 bakeries (1 with *Mucor* sp. resistant to voriconazole, another with *Chrysosporium* sp. resistant to itraconazole). Results demonstrate the importance of a detailed characterization of fungal burden since toxigenic and resistant species were isolated. The results claim attention for the possible presence of mycotoxins in bread as they resist to high temperatures. Azole-resistant species detected may have originated due to the use of triazole fungicides in cereals crops contributing to the development of multi-resistant fungal populations.

Keywords: Flour contamination, Portuguese bakeries, fungal burden, toxigenic fungi, mycotoxins

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Evaluation of analytical parameters for determination of bisphenol A in foods

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ABSTRACT

Bisphenol A is a chemical contaminant that interferes in the endocrine system and is present in polycarbonate plastic and epoxy resins used in food packaging. In child development, exposure to this endocrine disrupter may produce changes in estrogenic functions. According to Food and Agriculture Organization-FAO/WHO, there is a need to validate methodologies for the determination of bisphenol A concentrations in foods for children in countries outside North America and Europe. Thus, this work aims to evaluate analytical parameters for validation of methodology for the determination of bisphenol A by gas chromatography coupled to mass spectrometry. The influence of different temperatures (30, 60 and 80 °C) on the derivatization of the bisphenol A standard was evaluated. The standard was derivatized with a fixed volume of N,O-Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) for 30 min. The derivatization product (bis-trimethylsilyl-ether) was identified with a retention time of 17.28 min by selective ion monitoring (372 and 357m/z). Previous results confirm that the influence of the added BSTFA volume and the reaction time is small, however, the temperature is a determining factor in the derivatization efficiency. The best results were obtained at the temperature of 60 °C, which was used to prepare the standard curve. The analytical curve with derivatized standard (0.01-0.50 µg.mL⁻¹) was obtained (R²=0.98) for quantification and evaluation of the linearity of the method. The correlation coefficient value obtained was not considered satisfactory, but was expected since reduced concentrations were evaluated. Thus, new tests will be performed to evaluate the concentrations of the solutions and the response of the detector to obtain better results. From the elaboration of a new curve, it will be possible to establish the limits of detection and quantification, as well as the linearity interval, in order to quantify the levels of bisphenol A in infant foods.

Keywords: Food packaging, foods for children, gas chromatography

Assessment of ochratoxin A stability during dough fermentation and baking of maize bread

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ABSTRACT

Cereals are the first source of ochratoxin A (OTA) in the human and animal diet and maize and derivatives feature an outstanding place. Ochratoxigenic fungi and OTA in maize grains, in pre- and post-harvest, have been widely reported. OTA is a nephrotoxic, teratogenic and immunotoxic compound and it has been classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC). The aim of the present study was to assess the stability of OTA in two stages of maize bread making: dough fermentation and baking. This study was done by using maize flour free of OTA, which was spiked with 2, 5, and 10 µg of OTA/kg and mixed with sodium chloride, water and yeast cake (*Saccharomyces cerevisiae*) in suitable proportions. Dough pieces of 80 g were prepared. Fermentation was carried out at 29–30 °C, for 1 h and the baking conditions were: 190, 207, 223, and 240 °C for 50, 40, 35 and 30 min, respectively. OTA level were controlled after fermentation of the dough and after further baking at the different temperature–time combinations. OTA was extracted with acetonitrile/water (60/40, v/v), cleaned with immunoaffinity column (Vicam) and analyzed by LC-FLD. It was observed that under the assayed conditions, both fermentation and baking of dough have a significant effect ($p > 0.05$) on the reduction of the initial OTA level during the manufacture of bread ready for human consumption. No significant differences ($p > 0.05$) in the reduction of OTA in relation to the temperature/time combination was observed. Moreover, the reduction of OTA levels inside the bread was significantly lower than in the external surface.

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Keywords: Ochratoxin A, maize bread, dough fermentation, breadmaking, thermal treatment

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An automated methodology for the assessment of drinking water quality by monitoring priority substances at ultra-trace levels

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ABSTRACT

Safe drinking water is essential for public health, therefore the European Unión and the United States Environmental Protection Agency have established their respective regulations regarding drinking water quality. In general, drinking water is abstracted from different sources mainly groundwater and surface water. In this respect, with the aim of controlling and preventing contamination of aquatic ecosystems, the EU also introduced the Water Framework Directive, which establishes guidelines to control the pollution of surface water indicating a list of priority substances and setting out environmental quality standards for those chemicals. This list involves a high number of organic pollutants with restrictive maximum allowable concentrations including pesticides, PAHs or brominated diphenylethers (BDEs). So, this study was aimed at developing an automated, robust, reliable and highly sensitive and selective analytical method capable of simultaneously determining a broad range of organic pollutants at ultra-trace levels in drinking water. A total of 55 contaminants have been included in the method among which are pesticides, PAHs, BDEs and polychlorinated biphenyls (PCBs). The method involves the combination of on-line headspace solid phase microextraction (HS-SPME), ensuring the complete automation of the analyses, and gas chromatography coupled to magnetic sector high resolution mass spectrometry (GC-HRMS). Method validation showed good linearity ($R^2 > 0.99$), recoveries (80-120 %) and precision values ($< 20\%$) with quantification limits from 0.01 to 500 ng L⁻¹. Finally, the proposed method was successfully applied to 20 drinking water samples from Almería (South of Spain).

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Keywords: Organic pollutants, GC-HRMS, headspace-SPME

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Multiresidue analysis for pesticides and ochratoxin A determination in arabica coffee under different roasting levels

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ABSTRACT

Coffee is the largest agricultural commodity and the second trading in the world, such importance also makes coffee a very studied food matrix not only for its benefits but also for public health concerns. The use of some pesticides in coffee production systems are recognized to increase the productivity of this crop. The aim of this work was to quantify a pull of pesticides and ochratoxin A in five samples of Arabica coffee differentiated by their perceptual defective beans on it. Non-roasted (green coffee) and roasted in gradients (light, medium, dark, very dark levels) beans samples were subjected to quantitative pesticide and ochratoxin A analysis to monitor the possible degradation of these compounds during the roasting process. Pesticides and ochratoxin A were analyzed by a multiresidue LC-MS/MS and HPLC-FLD with immunoaffinity sample preparation, respectively. The study revealed the presence of three pesticide residues in five analyzed samples: i) flutriafol, used to control coffee rust, was found in two crude samples; ii) imidacloprid and iii) diazinon, were found only till medium roast. The found levels were 0.02 to 0.01 mg.kg⁻¹ (ppm). None of the detected pesticide residues exceeded the European Union (EU) Maximum Residue Limits (MRLs) for coffee beans that are 0.15 mg.kg⁻¹; 1.0 mg.kg⁻¹ and 0.05 µg/kg, respectively. Concerning ochratoxin A only a sample with more defective beans shows ochratoxin A in very dark roast, although in a very low level (0.06 µg/kg). In contrast, for the green sample with less defective beans ochratoxin A was not detected. The quantification values were 0.7 to 0.01 µg/kg. Ochratoxin A in all samples not exceeded EFSA MRL for roasted coffee (5 µg/kg).

Keywords: Mycotoxins, coffea arabica, insecticides, multi-detection approach



Development of practical ic-ELISA for aflatoxin tracking in chicken liver and egg

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ABSTRACT

Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) based on anti-aflatoxin B₁ monoclonal antibody (mAb) was standardized and validated for aflatoxin tracking in chicken liver and egg. The mAb was produced *in vitro* by cultivation of hybridoma strain AF.4 generated by Kawamura *et al.* (1988). The ic-ELISA was validated in liver with mAb concentration at 0.55 mg mL⁻¹ (titer of 1:10,000), whereas in egg it was 1.47 mg mL⁻¹ (titer of 1:30,000). Matrix interference was 9.8% in liver and 5.4% in egg, and both assays showed adequate linearity ($r^2 = 0.99$). Limit of detection and quantification in liver were 1.2 ng g⁻¹ and 1.5 ng g⁻¹, respectively; same data in egg were 0.35 ng g⁻¹ and 0.57 ng g⁻¹, respectively. Mean recovery rates in liver were 91, 91 and 81% spiking aflatoxin B₁ at 1.5; 3.0 and 5.0 ng g⁻¹, respectively; the recovery rates in egg were 96, 98 and 99% for 1.0; 2.0 and 5.0 ng g⁻¹. Precision was expressed by repeatability (CV= 4% and 12% for liver and egg, respectively), and intermediate precision (CV = 5% and 16%, for liver and egg, respectively). A total of 200 egg samples from producing local farms were analyzed by developed ic-ELISA. Aflatoxins were detected in 8.5% (n=17) samples at mean level of 0.8 ng g⁻¹ (ranging from 0.7 to 1.1 ng g⁻¹). Both developed ic-ELISAs could reduce the costly in approximate 160-fold factor when compared with commercial kits. Such rapid safe tracking attribute would be crucial to provide risk-free meat and egg in a country also emerging as an egg provider in addition to broiler, which is traditionally established in globalized world.

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Keywords: Immunoassay, mycotoxin, meat, poultry

Co-occurrence of zearalenone and deoxynivalenol in corn food products marketed in SerbiaLjilja Torovic^{1,2}**ABSTRACT**

Occurrence of zearalenone (ZEA) and deoxynivalenol (DON), secondary metabolites of some *Fusarium* fungi, was investigated in corn food products available on the Serbian market. A total of 56 samples was collected in 2015 and 2016 (14 and 32 corn flours; 7 and 3 corn flakes, respectively) and analysed by HPLC-FLD (ZEA) /-UV (DON), after preparation on immuno-affinity columns with monoclonal antibodies. In 2016, percentage of positive corn flour samples was two-fold higher for ZEA than for DON (75% vs. 37.5%), while co-occurrence was registered in 25% of the samples. Contamination levels ranged from 1.2 to 130 µg/kg ZEA (overall mean 7.4 µg/kg) and 25.2-327 µg/kg DON (overall mean 31.7 µg/kg). Only one sample containing ZEA at 130 µg/kg exceeded the maximum allowed level (ML). Results obtained in 2016 were compared with the ones from 2015 in terms of the following ratios: percentage of positive samples ZEA 0.81, DON 0.44, co-occurrence 0.29; mean level ZEA 0.17, DON 0.10; percentage of non-compliant samples ZEA 0.14, DON 0/7.1. Apparent difference in mycotoxin occurrence in two survey years was caused by the change of climate conditions in two corn growing seasons, affecting *Fusarium* growth and mycotoxin production. In case of corn flakes, ZEA was detected in all, and DON in one sample taken in 2016 (overall mean 3.0 and 12.3 µg/kg, respectively), but none of the samples surpassed the MLs. Comparison of the results for 2016 and 2015 showed the following ratios: percentage of positive samples ZEA 1, DON 0.66, co-occurrence 0.75; mean level ZEA 0.11, DON 0.06; percentage of non-compliant samples ZEA and DON 0/14.3. Substantially lower levels of ZEA and DON were found in corn flakes compared with flour samples, as could be expected due to processing effects. Mycotoxins occurrence in corn food products is of interest for regulatory purpose, as well as for population exposure assessment, especially for people on gluten-free diet with increased consumption of corn products.

Keywords: Zearalenone, deoxynivalenol, corn

**Aflatoxins and ochratoxin A in special types of flours:
a survey of the Serbian retail market**Ljilja Torovic^{1,2}**ABSTRACT**

A survey of the Serbian retail market was conducted regarding occurrence of aflatoxins (AFs) and ochratoxin A (OTA) in special types of flours in order to check compliance with food safety legislation and gather data for population exposure assessment. A total of 52 flour samples was collected in 2016 (32 corn and 20 buckwheat, rye, oat, barley, wheat (excluding white), rice and millet flours) and analysed using immunoaffinity chromatography on monoclonal antibodies and HPLC-FLD, with UV postcolumn derivatization for AFs. Survey revealed presence of AFs in 56.3% of corn flours: range 0.04-4.36 µg/kg, overall mean 0.49 µg/kg, one sample over maximum level (ML) for AFs (4 µg/kg), but three exceeded ML for AFB₁ (2 µg/kg). OTA was found in 40.6% of corn flours: range 0.08-7.16 µg/kg, overall mean 0.47 µg/kg, one sample over ML of 3 µg/kg. Co-occurrence of AFB₁ and OTA was recorded in 34.4% of corn flours. Regarding other types of flour, AFB₁ was detected only in one rye flour, while OTA showed wider occurrence: one out of four buckwheat flours, two out of eight wheat, and all three rye flours: 0.07, 0.40 and 23.0 µg/kg (7.7-fold over ML). Results of the 2016 survey were compared with the survey conducted in 2015 (33 flours analysed, 14 corn and 19 other cereals). In case of corn flours the following ratios were noticed: percentage of positive samples AFs 7.9, OTA 1.1, co-occurrence 4.8; mean level AFs 0.75 (AFB₁ 0.74), OTA 1.0; percentage of non-compliant samples AFs 0.4 (AFB₁ 1.3), OTA 0.4. Regarding other cereals, in 2015 AFs had been found in two rice flours (one above MLs for AFB₁ and AFs), while OTA was present in the same types of flour as in 2016, but there was no samples exceeding ML. In order to effectively safeguard health of the population, risk-based control system should be established in the framework of food safety in Serbia to prevent market release of the foodstuffs not in compliance with the legislation.

Keywords: Aflatoxins, ochratoxin A, flour

Aflatoxins production in fungal isolates of black-eyed bean (*Vigna unguiculata*)

Luciana Santa Barbara Bittencourt ¹; Leonardo Fonseca Maciel ¹;
Maria Spinola Miranda ¹; Tania Fraga Barros ¹

ABSTRACT

Some filamentous fungi produce mycotoxins, secondary metabolites detrimental to the health of humans and animals. The most studied mycotoxins are aflatoxins due to their toxicity and carcinogenicity. The black-eyed bean (*Vigna unguiculata*) is a legume of high nutritional value, which is part of typical Bahian dishes. The objective of this work was to analyze the fungal contamination index and aflatoxin production in fungal isolates of black-eyed bean, commercialized in Salvador city, Bahia, Brazil. Ten black-eyed bean samples were analyzed. To the mycological analysis was performed Sowing the grains in Sabouraud Dextrose Agar (SDA) plus 6% sodium chloride. The sowing was done in duplicate and the plates were incubated at room temperature for seven days. After incubation, the macroscopic characteristics of each colony were described and the identification of the fungus was performed through the microculture technique, in parallel, the fungi were preserved in distilled water and mineral oil. Aflatoxins (B₁, B₂, G₁ and G₂) were determined in the isolated fungi using immunoaffinity column (IAC) for extraction with subsequent analysis by thin layer chromatography (TLC) and reading under ultraviolet light at 366 nm. All samples showed fungal growth in less than seven days. *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Penicillium* sp., *Curvularia* sp. e *Fusarium* sp. were found in the analyzed samples. In the fungal isolates of *Penicillium* sp., *A. flavus* and *Curvularia* sp. the presence of aflatoxin B was identified.

Acknowledgements: The authors thank CAPES and CNPq (Brazilian Government Organizations) for grant aid and fellowship.

Keywords: Mycotoxin, extraction, aflatoxin B₁

***Alternaria* and ergot toxins – from pasture to plate?**Madeleine Gross ¹; Julia Irina Bauer ²; Ewald Usleber ²**ABSTRACT**

Considerable efforts have been made into the understanding and control of *Fusarium*, *Penicillium* and *Aspergillus* toxins. In contrast, knowledge on many aspects concerning *Alternaria* and ergot toxins is unsatisfactory. One aspect is the deficiency of data concerning sources of exposure of livestock, another one is the unknown fate of these toxins in the feed and food chain, including absence of carry-over studies. In this context, mycotoxinogenic endophytic fungi in pasture have to be considered. Ergot alkaloids may be produced by endophytic *Neotyphodium* spp. in grasses, *Alternaria* toxins may be produced by non-systemic endophytic strains of *Alternaria* ssp. in grasses and other plants used as feeds. Regional differences in prevailing climatic conditions may influence the interaction between fungal endophyte and plant host, including qualitative and quantitative patterns of mycotoxin production. However, the impact of mycotoxins in pasture on livestock feeding and transfer into food of animal origin is not well studied within Europe. Here we report the results of two pilot studies into this direction. Concerning *Alternaria* toxins, freshly cut grass, grass and maize silage for dairy cow feeding was comparatively analysed for alternariol by two immunoassay methods. All grass silages and the majority of the other samples were positive, up to mg/kg levels. Co-presence of yet unknown alternariol-like compounds seemed to be likely. Bulk milk from the same dairy farms was alternariol-negative, but this could not exclude the presence of metabolites. A second study aimed at ergot alkaloids in grasses in Germany. Again, the results obtained by immunoassay and by HPLC method showed frequent presence of ergot alkaloids at the mg/kg level. Overall, the results show that dairy cows and other herbivore livestock in Germany may be frequently exposed to *Alternaria* toxins and ergot alkaloids via pasture, which calls for further study into metabolism and carry-over into milk and meat.

Keywords: Feed, mycotoxin, milk, carry-over, food, alternariol, ergot alkaloids

Antifungal activity of phenolic extract obtained from rice bran fermented by *Rhizopus oryzae*

Taiana Denardi De Souza¹; Carlos Luz²; Fernando Bittencourt Luciano³;
Jordi Mañes²; Eliana Badiale-Furlong⁴; Giuseppe Meca²

ABSTRACT

The search for natural compounds that have antifungal activity against toxigenic strains present in foods is a current trend as several harmful effects have been related to the use of synthetic antifungals. Minimal Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of a phenolic extract obtained from rice bran fermented with *Rhizopus oryzae* CCT 7560 were evaluated against 7 *Fusarium*, 3 *Aspergillus* and 3 *Penicillium* mycotoxigenic strains. Rice bran was subjected to solid-state fermentation with *Rhizopus oryzae* CCT 7560 at 30°C for 24 h, using an initial population of 4×10^6 spores/g. The phenolic compounds present in the fermented biomass were extracted with methanol, clarified and lyophilized. Then, the extract was resuspended in water and total phenolic compounds were quantified spectrophotometrically by the Folin-Ciocalteu method. MIC and MFC evaluations were performed using microdilution method according to the Clinical Laboratory Standards Institute. Total phenolic compounds were present at 33.8 mg/g in the final extract. MICs for *Fusarium* strains were in the range of 3.9×10^{-2} - 7.8×10^2 mg/L, 3.9×10^2 - 3.1×10^3 mg/L for *Aspergillus* strains and 3.9×10^{-2} - 3.1×10^3 mg/L for *Penicillium* strains. The MFCs found for the phenolic extract were 7.8×10^2 - 1.6×10^3 mg/L, 7.8×10^2 - 6.3×10^3 mg/L and 7.8×10^2 - 6.3×10^3 mg/L for *Fusarium*, *Aspergillus* and *Penicillium* strains, respectively. Rice bran is a byproduct, which commonly has very low added value and is used as animal feed. Thus, the phenolic extract obtained from rice bran biomass may be a natural alternative for fungal inhibition in food products and in agriculture. Further studies will investigate the activity of this extract as an additive for bread and as a fungicide for corn.

Keywords: Minimum fungicidal concentration, minimal inhibitory concentration, toxigenic fungi

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Natural antifungals obtained from agricultural subproduct against strains of *Penicillium verrucosum* and *Aspergillus flavus*

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ABSTRACT

Phenolic compounds are being studied as a natural alternative in inhibiting the growth of toxigenic fungi that contaminate foods. In this work, phenolic compounds were extracted from rice bran biomass fermented by *Rhizopus oryzae* and applied under strains of *Penicillium verrucosum* and *Aspergillus flavus*. The fermentation of *Rhizopus oryzae* CCT 7560 was carried out in a solid state at 30°C, with initial spore concentration of 4x10⁶ spores/g of rice bran over 120 h. The free phenolic compounds from fermented biomass were extracted with methanol and clarified. After evaporation, they were dissolved in water and quantified spectrophotometrically by the Folin-Ciocalteu method. The phenolic acids of the extracts were identified and quantified in HPLC-UV. Strains of *Penicillium verrucosum* and *Aspergillus flavus* were incubated in PDA medium and after development of the cultures (7 days) mycelium (diameter 1 cm) discs were obtained. These were placed in the center of petri dishes containing PDA and phenolic extract (3327 ppm). In this form, they were grown at 25°C for 7 days. Potential inhibition was determined by measuring the halo (direct form) of fungal growth and by reducing the content of glycosamine, quantified through n-acetylglucosamine. The highest percentage of fungal inhibition was promoted by the phenolic extract obtained from 24 h fermented rice bran biomass with *Rhizopus oryzae*, which contained 3190 µg/g of free phenolic compounds. In this phenolic extract, the gallic acid is the major phenolic acid (720 µg/g). The inhibition indicated by the glucosamine content was 80.6% and 34.6% for *Penicillium verrucosum* and *Aspergillus flavus*, respectively. It has been demonstrated that the use of phenolic compounds extracted from natural source is a promising alternative to promote fungal inhibition without the risk represented by the synthetic active principles.

Keywords: Phenolic compounds, rice bran, toxigenic fungi

Analysis of organophosphorus pesticide residues in fruits extracted by QuEChERS and gas chromatography with nitrogen phosphorus detection

Virginia Cruz Fernandes¹; José Maria Oliveira¹; Valentina Fernandes Domingues¹; Cristina Delerue Matos¹

ABSTRACT

Introduction/Aims: Fresh vegetables, fruits and pulses are the important part of a healthy diet because of the presence of significant amount of nutrients and minerals in them. However, at the same time, they can also turn out to be source of toxic substances such as pesticides. Among various pesticide classes, organophosphorus pesticides (OPPs) group is the most widely used class of agricultural pesticides. The increasing public concern about the possible health risk of pesticide residues in the diet has profoundly modified crop production strategies with emphasis on food quality and safety. Apart from this, the wide spread concern for health of society has led to the strict regulation of MRL of pesticide residues in food. The analysis of OPPs is highly challenging due to the tendency of pesticides losses during the several steps of the methodology: sample preparation, clean up, storage of sample extracts and standard solutions as well as during GC analysis. Seeing the current need to address the awareness for long term moderate exposure of pesticides and high analytical requirements, the following study on evaluation of the different OPPs in red fruits will be performed using new strategies in analytical procedures.

Material and methods: The extraction of the OPPs was performed by using modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. Some extraction conditions such as salt addition, sample acidification, solvent volume and clean up step were evaluated. Different clean ups compositions were tested including with nanomaterials. The use of analyte protectants were also tested.

Results/Conclusion: The method proved to be simple and gave quantitative results for the assayed analytes, providing good validation parameters, such as linearity, limits of detection and quantification and precision. The nanomaterials showed good performance when applied as clean up. The preliminary results reinforce the relevance of this study for food chemistry namely food safety analysis.

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Keywords: Organophosphorus, QuEChERS, GC-NPD, nanomaterials

Validation of a SPE clean-up method for ochratoxin A determination in red wineAna Luísa De Sousa ¹; Armando Venâncio ¹; Luís Abrunhosa ¹**ABSTRACT**

The quality of Portuguese wines has improved considerably during the last decades as modern viticulture and enological practices were adopted. To maintain high quality standards it is also important to control any hazard that may jeopardize wine safety. One potential hazard for wines is the occurrence of the mycotoxin, ochratoxin A (OTA). This fungal metabolite occurs and exerts its toxic effects in small quantities, thus sensitive and reliable methods are required for monitoring its occurrence in foods. In order to minimize the interfering effect of the matrix and improve the selectivity and sensitivity of the analytical method, a concentration and cleaning step is often necessary. Solid phase extraction (SPE) is a technique with numerous advantages for that purpose. The objective of this work was to optimize and validate an analytical method for the determination of OTA in red wine using the SPE column Strata-X-A (Phenomenex). A not contaminated local red wine was fortified with OTA at concentrations of 0.05 to 10 µg/L, samples were clean-up using Strata-X-A columns in triplicate, analyzed by HPLC with fluorescence detection, and method recoveries, selectivity, stability, linearity, limit of detection (LOD), and limit of quantification (LOQ) determined. The method showed a linear response within the concentration range of 0.05 to 10 µg/L with a correlation coefficient of 0.9999. Within this concentration range, recoveries varied between 111% and 87%, respectively. The intra-day RSD was below 8%. The LOD and LOQ of the method was 0.005 and 0.015 µg/L, respectively. Portuguese red wines were analyzed using this method. Four wines did not reveal any OTA, and seven add OTA in concentrations that ranged between 0.02 and 0.441 µg/L. In conclusion, 20 years after the first studies reporting the presence of OTA in wines, levels of this mycotoxin in Portuguese wines are still low.

Keywords: Ochratoxin A, wine, solid-phase extraction

Influence of drying methods on cocoa (*Theobroma cacao* L.): occurrence of ochratoxin A

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ABSTRACT

Drying is responsible for the reduction of the acidity of the cocoa beans and must be conducted to obtain moisture content of approximately 7%. Excessive drying can make a shell brittle, while excess moisture favors fungal development. Ochratoxins are secondary metabolites produced by fungi from the genera *Aspergillus* and *Penicillium*, usually present in cereals, coffee, grapes, peppers and cocoa. Ochratoxin A (OTA) is known for its carcinogenic, nephrotoxic, teratogenic, genotoxic, and immunotoxic properties in animal cells. Data regarding the occurrence of OTA in cocoa and chocolate is limited, especially in terms of pre-processing steps. This work aimed to evaluate the performance of different drying methods to evaluate the occurrence of OTA in cocoa samples. The samples were dried using four dryer types: Dryer with stainless steel Platform and plastic roof with UV protection; Artificial Dryer, using wooden platform with artificial heat source; Traditional Dryer in barge with wooden platform and drying by direct sun light; Mixed Dryer with stainless steel platform and mobile plastic roof with UV protection for drying coverage and exposure to sun light. Drying time was seven days, following the procedures adopted for the farm, located in southern Bahia. Three samples before and after the drying process from each dryer type were analyzed. OTA was determined by ultrahigh-performance liquid chromatography with fluorescence detection. Among the 32 analyzed samples, only one showed natural contamination by ochratoxin A (7.1 µg.kg⁻¹). The European Community in the EC Regulation No. 1881/2006 of the Commission of 19/Dec/2006 does not establish OTA limits for cocoa. The National Health Surveillance Agency of Brazil (ANVISA) establishes a value of 10 µg.kg⁻¹ of OTA in cocoa. The only positive sample showed a value lower than that established by ANVISA.

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Keywords: Mycotoxin, chromatography, immunoaffinity

Simultaneous determination of 13 tropane alkaloids in botanical samples by liquid chromatography coupled to high resolution mass spectrometry (Orbitrap)

Ana Romera-Torres ¹; Roberto Romero-González ¹; José Luis Martínez-Vidal ¹; Jesús Marín-Sáez ¹;
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ABSTRACT

Tropane alkaloids (TAs) are a group of more than 200 secondary metabolites, which naturally occur in numerous plant families, mainly in *Solanaceae*. They are present in all parts of the plant, and have a high toxicity for humans and animals. TA rich plant material can be accidental or mistakenly present in different bulk commercial grains or herbal preparations, among others. Due to the risks to human and animal health, the European Food Safety Authority (EFSA) has established an Acute Reference Dose (ARfD) at 0.016 µg kg⁻¹ body weight (b.w.), expressed as the sum of atropine and scopolamine. Bearing in mind that more than 200 TAs are known, this ARfD does not express a real acute dose of these compounds. Therefore, the development of analytical methods for the simultaneous and sensitive analysis of TAs are needed in order to provide a valuable tool for the evaluation of the presence of these substances in botanical samples. In this study, a method for the simultaneous determination of 13 TAs in botanicals samples has been developed using liquid chromatography coupled to an Exactive-Orbitrap analyzer. Because of the variability of physic-chemical properties of TAs, as polarity or pK_a, developing a simultaneous extraction method of TAs was a challenge. Methanol/water/formic acid (75:25:0.4, v/v/v) was used as solvent extraction, followed by a solid phase extraction (SPE) step for the clean-up and preconcentration of target compounds. The validated method provided recoveries from 75-128 %, precision ≤ 26 % (except for apoatropine) and limits of quantification ≤ 20 µg/kg for all compounds. The method was applied to the analysis of eleven botanicals samples, obtaining concentration from 5 (apoatropine) to 4340 µg/kg (sum of physoperuvine, pseudotropine and tropine) in six positive samples.

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Keywords: Tropane alkaloids, LC-Orbitrap

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Determination of tropane alkaloids in millet and linseed by liquid chromatography coupled to exactive-orbitrap

Jesús Marín-Sáez ¹; Roberto Romero-González ¹; Ana Romera-Torres ¹;
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ABSTRACT

Some grains as millet and linseed can be contaminated by tropane alkaloids (TAs), because the *Solanaceae* plants family which produces these compounds usually grows next to this grain cultivations. These compounds have anticholinergic activity in animals but they are more toxic in humans because the presence of an esterase enzyme in animal, which efficiently biotransformed the compound. The principal TAs are atropine and scopolamine, although there are other for which there is scarce information. Therefore, the development of quick, easy and reliable analytical methods for their analysis at trace levels in food from plant origin is desirable. This study is focused on the development and validation of an analytical method for the simultaneous determination of a large range of TAs in linseed and millet samples. For this purpose a simple solid-liquid extraction method was optimized, employing an extraction solvent formed by a mix of methanol:water 0.5% acetic acid (2:1, *v/v*), followed by a strong cation exchange SPE preconcentration and clean-up stage. The compounds were eluted using methanol containing 3% of ammonium hydroxide solution (25%) solution. Then the extract was analyzed by high pressure liquid chromatography coupled to mass spectrometer Orbitrap analyzer (LC-MS-Orbitrap). The method was validated in linseed and millet samples, obtaining recoveries in the range of 67-101%, precision values $\leq 17\%$ and detection and quantification limits ≤ 2 and $3 \mu\text{g kg}^{-1}$ respectively. The developed method was applied to real samples of millet and linseed, being a millet sample positive in scopolamine, atropine, anisodamine, tropinone and littorine, finding scopolamine at concentration of $23 \mu\text{g/kg}$.

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Keywords: Tropane alkaloids, LC-Orbitrap

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Influence of composition and baking process in chemical indicators of Maillard reaction in biscuits

Maria Cristina Antunes¹; Cátia Sousa¹

ABSTRACT

Biscuits are popular cereal foods commonly consumed all over the world. The principal ingredients are wheat flour, fat, sugar and water. During biscuits baking occur several complex reactions that are responsible for the transformation that happen to the dough in this stage, formation of the structure and texture of the biscuit as well as surface colour development. Among these reactions, Maillard reaction (MR) is one of most important and occurs between the carbonyl group of a reducing sugar with a free amino group of amino acids or protein. The occurrence of MR in bakery products originates the formation of numerous compounds essential for the development of sensorial properties, such as flavour, surface colour and texture. However, the relevance of MR is not only related to the impact of Maillard reaction products (MRPs) in food properties but also its potential effect in health human. For example, it has been reported that some MRPs can act as antioxidants, particularly melanoidins, which can provide health benefits. However, beside to these positive effects, the occurrence of MR during food processing is responsible for the formation of potentially hazardous components such as acrylamide, furan and 5-hydroxymethylfurfural. The aim of this work was to investigate the effects of biscuits composition with different flour (wholemeal and wheat), sugars (white sugar, fructose, light sugar, golden and brown sugar) and baking conditions (conventional oven and microwave oven) on MR extent, through the evaluation of some markers associated to this reaction. The effect of domestic recipes preparation was studied to provide useful information for exposure assessment and advice for consumers.

Keywords: Bakery products, maillard reaction products (MRPs), wheat flour, sugars

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Development of a fast and green chromatographic method for the determination of decabromodiphenyl ether in fish tissues

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants used in several industrial and household products to reduce their flammability. However, their toxicity in human and wildlife organisms, which is mostly linked to endocrine disruption events, has been raising high legal and health concerns worldwide. Human exposure occurs mainly through dietary route, in particular from contaminated seafood consumption. Therefore, a large number of solvent-assisted analytical approaches have been developed to accurately determine minor amounts of these pollutants in seafood. However, most analytical procedures are time-consuming, expensive, and lead to high organic solvent consumption, thereby also causing additional damage to the environment. Hence, the present work reports the development and validation of a two steps method, using QuEChERS extraction followed by Dispersive Liquid-Liquid MicroExtraction (DLLME) concentration step for the analysis of decabromodiphenyl ether (BDE-209) in white seabream (*Diplodus sargus*) muscle, liver, brain and plasma. Instrumental analyses were performed on an Agilent Technologies 7890B Gas Chromatography (GC) system coupled to a 7000C GC/Mass Spectrometry (MS) Triple Quadrupole operating on negative chemical ionization mode. Statistical validation revealed a large linear working range and both repeatability and intermediate precision presented low Relative Standard Deviations (RSDs, <10%). Method sensitivity was also confirmed and the accuracy was tested by recovery tests, both showing highly satisfactory results for all matrices. Therefore, the method developed proved to be effective for routine analysis and versatile for allowing the analysis of different matrices, including those with high lipid amounts. Moreover, it resulted in a substantial reduction in total analysis time and organic solvent consumption, thus embracing the green chemistry guidelines.

Keywords: PBDE, GC-NICI-MS, food safety, brominated flame retardants, seafood

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Enantiomeric degradation of quizalofop in soils by high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

Quizalofop-P is a systemic herbicide, absorbed by the leaves with translocation throughout the plant. This compound is the acid ester of quizalofop which is the parent compound, and its use as pesticide has not been approved, but its metabolites (quizalofop-p-ethyl, quizalofop-p-terfuryl and propaquizafop) can be used as phytosanitary product. During the degradation process of these metabolites, quizalofop could be formed into its two enantiomers, where quizalofop-p (*R* enantiomer) is the main active substance. Up to now, the maximum residue limit (MRL) in food commodities of quizalofop-p includes the parent compound, quizalofop, which has not been authorized as herbicide. It should be noted that although quizalofop has not been authorized it appears when phytosanitary products degrade into quizalofop-p. For these reasons, it was necessary the development of an analytical method for the monitoring of the enantiomers *R* and *S* of quizalofop. In the present study, the enantiomers of quizalofop were separated on Chiralpak AY3 chiral column by high-performance liquid chromatography using isocratic mode, utilizing ethanol 0.1% formic acid as mobile phase. Compounds were detected by mass spectrometry. A laboratory degradation study was performed evaluating three commercial products whose main components were propaquizafop, quizalofop-p-ethyl and quizalofop-p-terfuryl. Soil samples were spiked and the concentration of both enantiomers was monitored till 80 days after compound addition. The results of the degradation showed that the metabolites dissipated in soils to enantiomer *R* or *S* of quizalofop and the ratio between enantiomer *R* and *S* does not depend on the day and the amount of commercial product added to the soil. For quizalofop-p-ethyl and propaquizafop the percentage is 96% of enantiomer *R* and 4% of enantiomer *S*, whereas for quizalofop-p-terfuryl is 85% of enantiomer *R* and 15% of *S*, obtaining higher percentages of this isomer than with the other products.

Keywords: Enantiomeric separation, UHPLC-Orbitrap-MS, herbicides

Influence of raw materials and process variables on 3-Monochloropropane-1,2 Diol (3-MCPD) content in bakery productsSofia Oliveira ^{1,2}; Marisa Castro ^{2,3}**ABSTRACT**

3-Monochloro-1,2-propanediol (3-MCPD) and glycidol are contaminants in processed food, which have been well known for 30 years. The accepted daily intake (TDI) for 3-MCPD and its esters was reduced from 2 to 0.8 micrograms per kilogram of body weight per day ($\mu\text{g}/\text{kg bw}/\text{day}$). The present study was focused on identifying the possible causes leading to the level of 3-MCPD found in bakery products in response to changes in the raw materials used and process variables applied. A GC-MS method was developed and applied for 3-MCPD quantification. Two types of derivatizing agents (HFBA and BSTFA) were tested and compared. BSTFA was found to be more appropriate. A calibration was also performed, where a limit of quantification of 1ppb was obtained. In the pilot laboratory, 46 cake recipes from 4 references were analysed to determine the main cause to the levels of 3-MCPD found in the finished product. During these tests, the influence of glycerine, palm oil, salt and chlorine contents, the type of flour and the respective emulsifiers and flavorings were investigated. These variables were selected based on historical data. Dedicated analyses of some major types of raw materials, such as cocoa, were also elaborated. It was concluded that even if a particular raw material is the main cause of the levels of 3-MCPD found in a cake sample, feasible alternatives for replacement may not be possible, since the finished product may not present the quality characteristics expected by the customer. The study in toasts focused on the raw material present in the formulation that most influences the presence of 3-MCPD, the palm oil. Vibrational spectroscopies were used trying to monitor the presence of the contaminants in fat despite the strong chemical resemblance in their chemical structures. In conclusion, factors, such as the presence of chlorine, glycerin, palm oil (or its derivatives) and the high salt content, which can be considered critical, require further investigation.

Keywords: 3-Monochloropropane-1,2 Diol (3-MCPD), bakery products

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Mycotoxins in coffee – what reality?

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Robert Kosicki³; Jan Grajewski³

ABSTRACT

Coffee is a valuable product due to the still increasing demand for production and consume. Brazil, Vietnam and Columbia are the bigger producers and Finland, Norway and Netherlands are the countries with higher rates of consumption.

Unfortunately, it is subjected to various contaminations that can have an important impact in consumer's health and economy. The major compound of concern is ochratoxin A (OTA) but other mycotoxins can be present. However, roasting process has an important role in reducing mycotoxins presence, depending upon the combination of time and temperature. Since each European country applies different roasting conditions, significant differences in mycotoxins concentrations occur across the world even when the origin of the coffee beans is the same. Considering the above the aim of this study was to analyze mycotoxins (OTA, aflatoxins (AF) and citrinin (CIT)) in coffee ready to be consumed from different markets and different brands: Portugal (6), Austria (3), United States (2) and Timor (1). Twelve samples were collected from different types of brands sold as roasted beans. The extraction of OTA, CIT and AF was done on the immunoaffinity column Ochraprep (R-Biopharm), CitriTest HPLC and AflaTest WB (Vicom), respectively. Mycotoxins were analyzed with HPLC-FLD (Merck-Hitachi).

CIT and AF were not detected. OTA was detected in all the samples from Austria (2 results < 0.4 µg/Kg and 0.43 µg/Kg). In one of the Portuguese samples it was detected OTA (< 0.4 µg/Kg). Since the coffee beans have probably the same origin, it seems that indeed the roasting process adopted in each country and coffee industries influence the presence of mycotoxins. Further studies should be developed aiming to analyze other mycotoxins considering the possible climate changes influence. Additionally, a study should be developed to allow identifying how the roasting conditions adopted in each European country impact the presence of mycotoxins in coffee.

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Keywords: Coffee, mycotoxins, roasting conditions

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**Fungal flora from Tunisian olives carposphere:
diversity and volatile organic compounds**Hend Bejaoui ¹; Guido Flamini ²; Paula Rodrigues ³; Nourhène Boudhrioua Mihoubi ⁴**ABSTRACT**

Plant phyllosphere is an enormous environment on Earth densely colonized by microorganisms. Those microbiomes play essential roles in processes related to plant development and pathogen defense. They release different metabolites working as interspecies messages in the environment. Volatile organic compounds are among those released messages. Among the worldwide phyllosphere, oliveyards are widely spread in the Mediterranean basin. Like all foods, olives and oil quality begin in the field. Fungal microbiomes through their interactions with olives trees could have an impact on oil quality. In this work we were interested on fungal microbiomes from olives carposphere. Fifteen Tunisian fields from four climatic regions were studied. Fungi were isolated and identified to species with microscopic and molecular techniques. Their aromatic profiles were analysed by solid-phase micro-extraction (SPME) coupled to gas chromatography and mass spectrometry. Results showed that genus *Penicillium* (*P. polonicum*, *P. crustosum* and *P. expansum*) was predominant. The major volatiles identified were: Styrene, 1-octen-3-ol, 3-octanol, 3-octanone, 1,8-cineole. Styrene is a volatile hydrocarbon reported as 80 times more toxic than the volatile phase of toluene. The following three are oxylipins. They play essential roles in fungal morphogenesis and pathogenesis and are reported as metabolites with musty and earthy characteristics able to induce mycotoxin production. The last one is a terpene with a eucalyptus herbal camphor odor note. Could those volatiles be found on olives and olive oils? Could they impact chemical and sensory olive oils qualities? Could they induce mycotoxin production in olive oils? All those questions still to be answered...

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Keywords: Oliveyards, fungal microbiomes, Penicillium, oxylipins

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Analysis of elemental impurities in dietary supplements for weight loss and assessment of potential risk to human health

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Luísa L. Gonçalves^{1,2}; José Brito^{1,2}

ABSTRACT

Dietary supplements for weight loss are one of the best-selling products outside the pharmaceutical distribution network. They often contain plants in their composition, which are known sources of toxic elements (Rebiere et al. 2012; Zin 2014). The aim of this study was to compare the concentration of elemental impurities (Cr, Cu, Ir, Mn, Mo, Ni, Os, Pb, Pt, Rh and Ru) found in dietary supplements for weight loss with specified limits set by international bodies and to assess the cumulative non-carcinogenic risk to human health due to exposure to mixtures of such elements.

Materials and Methods: In this study, 25 dietary supplements for weight loss randomly purchased in 5 different suppliers in Portugal were analysed by Wavelength Dispersive X ray Fluorescence (WDXRF) spectrometry (Figueiredo et al. 2016). Risk assessment was performed according to Environmental Protection Agency (EPA) guidelines, through the Hazard Index (HI) (U.S. EPA 2001).

Results and Conclusions: Two of the analysed supplements show concentrations of Cr above EMA limit (25µg/g). One product contains a Mn concentration which is almost four times greater than EMA limit (250µg/g) and a Pb concentration which doubled the USP limit (1µg/g). Unconformities were detected between the labelled and the determined values. Despite the obtained HI (1.1E-1) reveals no potential risk of non-carcinogenic effects to human health, humans are often exposed by different sources and/or routes to toxic metals; thus, the additional consumption of these products cannot be ignored.

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Keywords: Elemental impurities, risk assessment, dietary supplements

**Selective *in vitro* binding of aflatoxin B₁ by
*Lactobacillus acidophilus***

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ABSTRACT

In the present study, we aimed to assess the ability of *Lactobacillus acidophilus* to remove aflatoxin B₁ (AFB₁) and ochratoxin A (OTA) from phosphate-buffered saline (PBS) under different conditions. Residual mycotoxin amounts were analysed using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The strain tested selectively removed AFB₁ from the medium with significant differences depending on incubation time and initial bacterial biomass. Removal of AFB₁ was a slow process and maximum reduction of 57.6% of AFB₁ was achieved after 72 h of incubation, at initial bacterial biomass of 3×10^{10} UFC/mL. While AFB₁ was significantly reduced in moderate amount (36-57%), no ochratoxin A amounts were removed. The strain in viable and non-viable (heat-treated) forms was also tested to remove mycotoxins. No significant differences in removal were found between viable and heat-treated bacteria. Moreover, the amount of AFB₁ removed by *Lactobacillus acidophilus* increases with increasing AFB₁ concentration and a binding mechanism has been proposed. These results indicate that *L. acidophilus* efficiently binds aflatoxin B₁. This ability of *L. acidophilus* should be applied to the removal of aflatoxin B₁ and further researched for commercial application in dairy products and feeds.

Keywords: Aflatoxin B₁, ochratoxin A, *Lactobacillus acidophilus*, *in vitro*

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Anti-aflatoxigenic effect of organic acids produced by *Lactobacillus plantarum*

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ABSTRACT

Molds play an important role in food spoilage, being estimated that 5 to 10% of the world food's production is lost due to fungal contamination. Further, certain fungal species produce highly toxic metabolites, designated as mycotoxins. Biopreservation, defined as the control of one organism by another, has received much attention in recent years. Also, some strains of lactic acid bacteria (LAB) that demonstrated antifungal and antimycotoxin properties gained interest to be used as natural biopreservatives. In this work, it is shown that the cell free supernatant (CFS) of *Lactobacillus plantarum* UM55 inhibited the growth of aflatoxigenic fungi, *Aspergillus flavus*, by 32% and the production of aflatoxins (AFs) by 91%. These inhibitions were lost when the CFS pH was neutralized. Additionally, it was observed an increase of the inhibitions with increasing concentration of CFS. Other aflatoxigenic strains, such as *A. parasiticus*, *A. arachidicola*, *A. nomius* and *A. minisclerotigenes* were inhibited by the CFS of the bacterium in different extents. Organic acids present in CFS were quantified, with main differences between CFS and control found in the levels of lactic acid, phenyllactic acid (PLA), hydroxyphenyllactic acid (OH-PLA) and indole lactic acid (ILA). When tested, individually against *A. flavus*, all the compounds were able to inhibit fungal growth and AFs production. PLA showed the stronger effects and the 90% inhibitory concentration (IC₉₀) for fungal growth and AFs was of 11.9 and 0.87 mg/mL, respectively. AFLs IC₉₀ for ILA, OH-PLA and lactic acid were of 1.47, 1.80, and 3.92 mg/mL, respectively. Inhibitory effects of *L. plantarum* UM55 seems to be related to the production of lactic acid, PLA, OH-PLA and ILA.

Acknowledgments: Ana Guimarães received support through grant SFRH/BD/103245/2014 from the Portuguese Foundation for Science and Technology (FCT). Luís Abrunhosa was supported by grant UMINHO/BPD/51/2015 from project UID/BIO/04469/2013 financed by FCT/MEC (OE). This study was supported by FCT under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684); of BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte; and under the scope of the projects RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462).

Keywords: Lactic acid bacteria, aflatoxin, organic acids, Lactobacillus plantarum, biopreservation

Antifungal effect of organic acids from lactic bacteria on *Penicillium nordicum*

Ana Guimarães ¹; Armando Venâncio ¹; Luís Abrunhosa ¹

ABSTRACT

Lactic acid bacteria (LAB) have been gaining attention for the antifungal properties of some strains. The control of fungal growth is especially important since moulds are responsible for significant spoilage of food and feed. Additionally, they are able to produce toxic compounds known as mycotoxins that cause serious health hazards. Some LAB strains have the ability to inhibit fungal growth and also the production of mycotoxins. In this work, cell free supernatants (CFS) of *Lactobacillus plantarum* UM55 and *Lactobacillus buchneri* UTAD104 were tested for the inhibition of *Penicillium nordicum* growth and ochratoxin A (OTA) production. Fungal growth was inhibited in only 18 and 11% by CFS of *L. plantarum* and *L. buchneri*, respectively. However, the production of OTA was reduced approx. in 70% by both strains. In order to determine the nature of compounds responsible for this activity, CFS were subjected to heat, proteases and neutralization of pH. It was observed that CFS retained its inhibitory properties after being autoclaved and treated with proteases. However, when submitted to pH neutralization, CFS lost its activity. Some organic acids produced by these LAB strains were also tested for their inhibitory capacity. Calculation of inhibitory concentrations shown that butyric and propionic acids were the most effective in inhibiting *P. nordicum* growth and OTA production, followed by indole lactic acid (ILA), phenyllactic acid (PLA), acetic acid, hydroxyphenyllactic acid (OH-PLA) and lactic acid. CFS were analysed by HPLC-PDA for the quantification of those organic acids. For *L. plantarum* UM55 main differences were found in the levels of lactic acid, PLA, OH-PLA, and ILA. For *L. buchneri* UTAD104, levels of acetic, lactic and PLA were higher than in the control experiment. In conclusion, ability of LAB to inhibit mycotoxigenic fungi depends of strain capability to produce certain organic acids, and those acids may differ from strain to strain.

Keywords: Lactic acid bacteria, *Penicillium nordicum*, ochratoxin A, organic acids



Potassium food sources in Portuguese diet

Ana Nascimento ¹; Susana Santiago ¹; Mariana Santos ¹; Maria Antónia Calhau ¹

ABSTRACT

Potassium is an essential nutrient involved in fluid, acid and electrolyte balance and is required for normal cellular function. Dietary deficiency of potassium is very uncommon due to its widespread occurrence in foods. Potassium is classified as a nutrient of public health concern, because its under consumption has been linked in scientific literature to adverse health outcomes. The aims of this study were to assess the potassium food sources and evaluated the Na/K ratio for the food groups under study. Samples were select according to the Total Diet Study (TDS), including core foods representing the overall diet of a population while covering specific foods containing high levels of chemical substances under review. Foods were collected according to a sampling plan, then were prepared as consumed and pooled before analysis. Analysed samples (111) were grouped according to food classification system FoodEx2 level 1: Composite dishes and soups; meat, eggs and dairy; fish and seafoods, vegetables, legumes and cereals; fruits and confectionery. Analyses were carried out in accordance with ISO standard 17025. Sodium and potassium levels were determined using an Inductively Couple Plasma Atomic Emission Spectrometry–ICP-OES. Sodium and potassium contents ranged from: 8.99-1476 mg/100g in dried figs and lupines and from 7.63-1583 mg/100g in lupines and fresh codfish, respectively. The ratio Na/K presented lower values in fruits in general, dried figs, raisins, unprocessed meat, vegetables and legumes. Higher values of Na/K ratio were found in lupines, ham, sausages, olives, squid, clams and cheese. Lowering the dietary sodium-potassium ratio by increasing the consumption of potassium-rich foods, like fruits in general, legumes and vegetables as shown in our results can be a useful component of dietary advice. Increases in dietary potassium have been shown to delay the incidence of hypertension. Also, potassium-rich foods in general presented lower amounts of sodium.

Keywords: Potassium, Na/K ratio, public health

Microbiological contaminants in raw salads

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ABSTRACT

The search for a better quality of life combined with a healthy diet has led to the emergence of vegetable consumption in a variety of ways throughout the world. Fruits and vegetables are necessary ingredients in a healthy and balanced diet, providing health benefits in addition to satisfying dietary needs. Salads represent whole meals that can include a variety of ingredients such as raw vegetables, cooked meat and pasta, making them increasingly popular products among consumers. However, green vegetables were identified as the group of products of greatest concern from a microbiological safety perspective, since they are mostly consumed without any kind of thermal processing. Following, the objective of this study was to evaluate the presence of microbiological contaminants in samples of uncooked salads consumed in different commercial establishments in the state of Bahia. For this purpose, 533 salads samples from different commercial establishments were evaluated for the most probable number (MPN) of coliforms and *Salmonella* spp. Was determined according to the methodology described by the American Public Health Association - The data obtained experimentally showed that of the 533 samples analyzed, 170 (31.8%) presented coliforms, using the standards suggested by RDC No. 12, 2001 (ANVISA - BRAZIL) above the maximum limit of 10^2 NMP / 25g determined by legislation, while in four of them (0.75%), were detected positive for *Salmonella* spp. The results obtained evidence necessary for the implementation of good practices in the preparation and storage of salads, besides alerting the competent agencies regarding the intensification of inspection and promoting food safety.

Keywords: Coliforms, food safety, Salmonella

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Cytotoxicity effects of bisphenol A in Hep2 and MRC5 cell lines – Is established TDI protective enough?

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ABSTRACT

Endocrine disrupting chemicals (EDCs) are exogenous compounds generally employed in the food and drinks processing and packing, with potential associated hazardous effects for human health. Bisphenol A (BPA) stands out as a paradigmatic xenoestrogen for which human exposure is widespread, omnipresent and persistent, particularly through ingestion of contaminated food and beverages. European Food Safety Authority (EFSA) experts on the latest comprehensive re-evaluation of BPA exposure and toxicity reduced the tolerable daily intake (TDI) from 50 µg/kg bw/day to 4 µg/kg bw/day. This compound is included in the Priority List of Chemicals within the EU Strategy for Endocrine Disruptors and classified as high concern in terms of human/wildlife exposure. BPA even at very low concentrations can influence cell fate in a cell type specific manner and epidemiological studies have demonstrated positive correlations between BPA levels detected in human biological samples and etiology of numerous pathologies. Here we aimed to evaluate potential cytotoxic effects of BPA concentrations within the range detected in human biological samples consistent to exposure levels below the established TDI, namely 4.4 µM, 4.4 nM and 0.44 nM in Hep2 and MRC5 cell lines. Cytotoxic effects were assessed through the fluorometric method CellTiter-Blue assay and expression of BPA high affinity receptor GPER, in MRC5 cells, was performed through immunofluorescence technique. We demonstrate that MRC5 cells express GPER receptor and no significant differences in viability associated with BPA exposure was observed in both cell lines. Our results indicate that BPA concentrations below the EFSA established TDI do not affect Hep2 or MRC5 cellular viability which sustains the indication of safe levels. Nevertheless, further research must be performed in order to assess potential effects that are not reflected by cytotoxicity such as genotoxicity and mixtures interactions.

Keywords: Bisphenol A, human exposure, cytotoxicity effects

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Mycotoxins adsorption by microorganisms isolated from Kefir grains

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ABSTRACT

A novel alternative for mycotoxins decontamination is the use of microorganisms that bind mycotoxins and reduce their gastrointestinal absorption. Lactic acid bacteria and yeasts were isolated from a Kefir culture and evaluated for their mycotoxin adsorption and biotransformation ability. Strains with high binding ability were identified based on DNA sequencing. The binding stability was determined by washing the complexes microorganism/mycotoxin with buffer solutions to simulate the pH conditions in the gastrointestinal tract. The results indicate that the microorganism consortium of Kefir grains adsorbed 82 to 100% of aflatoxin B₁ (AFB₁), zearalenone (ZEA) and ochratoxin A (OTA) when cultivated in milk. The most effective strains in adsorbing the mycotoxins were identified as *Lactobacillus kefir*, *Kazachstania servazzii* and *Acetobacter syzygii*. The strains *L. kefir* KFLM3 was able to adsorb 80 to 100% of the mycotoxins when cultivated in milk. However, desorption experiments showed that yeast *K. servazzii* KFGY7 retained more mycotoxin (65, 69 and 67% for AFB₁, OTA and ZEA, respectively) in the cells. Our findings revealed that kefir consumption can possibly reduce gastrointestinal absorption of these mycotoxins and consequently reduce their toxic effects. These Kefir isolates are promising for the development of fermented dairy products for human consumption.

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Keywords: Kefir, adsorption, mycotoxins, *Lactobacillus kefir*, *Acetobacter syzygii*, *Kazachstania servazzii*

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Evaluation of microbiological risk factors in ready-to-eat packaged salads

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ABSTRACT

Ready to eat vegetables (REV), could be fresh-cut fruits and vegetables, which have been physically altered in its original form, this is, peeled, cut and packaged. The safety and microbiological quality of REV, has been a concern in the last decades due to increased association with foodborne disease outbreaks related to consumption of raw vegetables. Green leafy vegetables seem to be the most frequently involved products. The main challenge is to find conditions in which microbial proliferation is delayed, for the maintenance of the quality and shelf life of the product. The objective of this study was to evaluate the risk factors to human health related with the consumption of REV. A total of 45 packed samples of REV where analysed, containing one type of vegetable (lettuce type Iceberg), 3 types (endive lettuce, radicchio lettuce and canons) and 4 types (pea leaf, red oak, canons and chives). Exhibitors at the point of sale, were assessed through a checklist (checklist) and the temperature of the packed product was measured. The results microbiological were: aerobic total counts, $4,7 \times 10^4$ to $5,7 \times 10^8$ ufc/g; *Enterobacteriaceae*, $9,0 \times 10^1$ to $8,9 \times 10^6$ ufc/g; *Escherichia coli*, <10 ufc/g; Molds, <10 to $2,4 \times 10^3$ ufc/g; Yeasts, <10 to $8,2 \times 10^4$ ufc/g and *Pseudomonas* spp., $6,0 \times 10^4$ to $2,2 \times 10^8$ ufc/g. In terms of prerequisites most of the equipment was in good condition. It was observed that 50% of the samples had higher temperatures ($7,8^\circ\text{C}$) above the limits considered ideal (0 to 4°C). Regarding the microbiological final acceptance, were classified as “Not satisfactory” 12.5% of the samples with 1 type of vegetable, 93.3% of the samples with 3 type of vegetable and all samples with 4 types of vegetable. These results indicate that the microbiological quality of these products need to be improved, and the greater the variety of leaves, the worse the microbiological quality. The high exposure product temperatures, show an important risk factor.

Keywords: Ready to eat vegetables, microbiological, control

Risk assessment of mycotoxins in buckwheat grain and its products

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ABSTRACT

The United Nation's Food and Agriculture Organisation (FAO) have indicated that the nutritional value of cereal crops and their products is changing due to the climate change. This results in increasingly higher mycotoxin contamination of agricultural produce. Although buckwheat (*Fagopyrum esculentum* Moench) grain and its products are highly valued in the global healthy food market, few studies have addressed the issue of mycotoxin contamination in them. The current study was aimed to quantify mycotoxins deoxynivalenol (DON), T-2 toxin, aflatoxin B₁ (AFB₁), ochratoxin A in buckwheat grain and its products in 2013–2015. Research evidenced that buckwheat grain was least contaminated with the investigated mycotoxins in 2013. Hot weather and drought during the buckwheat flowering and maturity stages in 2014 and 2015 impaired crop growth and development. All buckwheat grain samples were found positive for AFB₁. An especially high AFB₁ content (1.7–71.6 µg kg⁻¹) was in the grain harvested at BBCH 77 stage, while in the fully mature grain it was several times lower; however, it exceeded the EU allowable limit (2.0 µg kg⁻¹). These findings question the quality of buckwheat products. Hulls were 10-fold more contaminated than grain and residue (~0.60 µg kg⁻¹) of AFB₁ was found in flour. Groats, prepared without hot steam treatment contained 2.3±0.6 µg kg⁻¹ of AFB₁. Mycotoxin contamination of buckwheat grain is likely to have depended on the weather conditions, which were uncharacteristic of Lithuania's climate in experimental years (2014 and 2015). A trend showing that phenolic compounds might partially prevent contamination of buckwheat grain with trichotecene mycotoxins was revealed: grain and hull samples containing the highest concentrations of rutin and quercetin were significantly ($P < 0.05$) less contaminated with DON and T2.

Keywords: Buckwheat, mycotoxin, contamination, weather conditions

Effect of the application of oenological products on fumonisin B₂ (FB₂) reduction/removal in contaminated red and white wines

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ABSTRACT

Fumonisin is a mycotoxin produced by species of *Fusarium*, mainly *F. verticillioides* and *F. proliferatum*. Fumonisins have hepatotoxic and nephrotoxic effects in various animals and are also associated to human esophageal cancer [1] thus being classified by the IARC in Group 2 [2]. Chemically, fumonisins are characterized by a 19- or 20-carbon aminopolyhydroxyalkyl chain that is diesterified with propane-1,2,3-tricarboxylic acid groups [3]. Sharing a basic structure, several related groups of fumonisins have been isolated and identified (A, B, C and P). Fumonisins B (FBs) are the major forms found in most food products. Recently they were detected in grapes, musts and wines around the world. It was established an association between *Aspergillus niger* and the presence of FB2 in grape must and wine. WHO has recommended a maximum tolerable daily intake of 2 mg/kg of body weight to FB1, FB2 and FB3, alone or in combination [4], therefore it is important to prevent and control its occurrence, as well other mycotoxins, in wines [5]. The main objective of this work was to evaluate the ability of different oenological products on FB2 removal of white and red wines. For this purpose, ten commercial oenological fining agents (mineral, synthetic and organic - proteins of animal and vegetable origin) were studied to remove FB2 in white and red wines artificially contaminated with FB2. In addition to the FB2 removal, the effect of these products on wine physicochemical characteristics, namely, flavonoids, non-flavonoids and total phenolic compounds were evaluated. In red wine all products showed low reduction on FB2 removal, being the highest value (30%) achieved by calcium bentonite. The results obtained by the action of these products may be considered as a pioneer approach on wine FB2 reduction/removal, with relevance for wine industry, in order to select the best fining agent to reduce toxicity and consequently to improve wine quality and safety.

Keywords: Fumonisin B₂, oenological products, wine

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Microbiological evaluation of gourmet meals for babyLúcia Matias ¹; Cátia Morgado ²; Carlos Brandão ²**ABSTRACT**

Babies constitute a risk group, hence the importance of assessing exposure to food contaminants. There is currently a high and increasing consumption of ready-to-eat meals (V gamma), and variations have appeared for the gourmet feeding for babies, so it became pertinent to carry out a study that allowed to evaluate and to analyze the risk factors present in this type of meals. 52 packaged meals for the final consumer were randomly collected for microbiological analysis, of which 17 were vegetable soups, 16 meals with rice and 19 meals without rice, and none of the meals contained raw ingredients. The samples were microbiologically analyzed in relation to; Total colony counts (TCC), Enterobacteria, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogens* and *Clostridium perfringens*. The samples were classified as Satisfactory (S), acceptable (A) and unsatisfactory (U). Concerning the research of *L. monocytogens* and *C. perfringens*, all samples presented negative results. Regarding the TCC the results ranged from 0 to 5,99 log/g, with a median of 2,79 log/g, for Enterobacteria from 0 to 4,38 log/g cfu/g, with a mean of 3,24 log/g., and *B. cereus* from 0 to 3,32, with a mean of 2,11. *E. coli* was present in 8% (4) of the samples, and ranged from 0 to 3,38 log/g with a mean of 1,72 log/g. Regarding the level of S/A/U of the total sampling, the results were 33%/29%/38%, and per parameter were; TCC – 33%/40%/ 27%, Enterobacteria – 77%/2%/21%, *E. coli* – 92%/0%/8% e *B. cereus* – 85%/11%/4%. Rice meals had a higher U level regarding *B. cereus* – 13% vs 5%. The results obtained point to a deficient hygienic and sanitary quality of these meals, with the aggravation of *B. cereus* presenting only 85% of A samples, which can have consequences in case of poor storage. We believe that the cooking process should be optimized and validated, because at the moment these meals do not present safety conditions to be consumed by babies.

Keywords: Baby, meal, hygienic, sanitary, microbiological

Importance of microbial culture collections for food safety research in climate change scenario

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ABSTRACT

Culture collections (CC) of microorganisms can play an important role in ensuring safe food for the world's population. The preservation of different groups of microorganisms such as astoxigenic, pathogenic, phytopathogenic, deteriorating or biotechnological, allows the development of research for their control or use. Then, it is important that these CC use appropriate preservation techniques, species identification criteria rigid and a multidisciplinary specialists group. The Microorganisms Culture Collection of the Department of Food Science (CCDCA / UFLA) of the Federal University of Lavras (Minas Gerais-Brazil) has been developing research projects that value and demonstrate the importance of CC for food safety. Above all, among these researches, the most important ones are: 1) Use of essential oils of medicinal plants and condiments in the control of toxigenic fungi; 2) Development of antiseptic with essential oils to control contaminating microorganisms from the hands of food handlers; 3) Monitoring of ochratoxigenic fungi in fruits and beans coffee; 4) Fungi prevalent in regional artisanal cheeses in Brazil; 5) Fungi and yeasts of the *terroir* microbiota of tropical wines; 6) Evaluation of the expression of genes involved in the synthesis of ochratoxin A as a function of the temperature changes in coffee fruit processing. In projects 3) to 6), climate change certainly will influence food safety. Therefore, CCs are important sources of research to ensure the preservation of microbial biodiversity, and the development of research for production of safe food even in the face of climate change different scenarios. The CCDCA / UFLA is accredited as a Faithful Depositary is a member of the WFCC and registered in the WDCM with the number 1081 and currently has more than 800 strains preserved at -80 °C, belonging to the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Talaromyces*.

Financial Support: FAPEMIG, CNPq.

Keywords: Microorganisms, preservation, biodiversity, culture collections

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Assessment of human health risk of toxic elements through the consumption of cinnamon in the diet

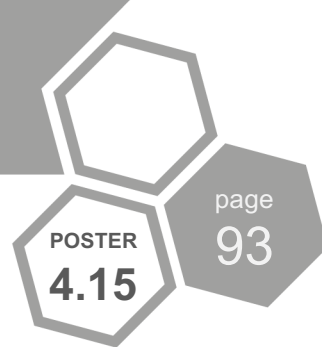
M. Alexandra Bernardo ¹; Tânia Fernandes ¹; João M. Maia ²;
Maria Fernanda Mesquita ¹; José Brito ¹; Luísa L. Gonçalves ¹

ABSTRACT

Among many pharmacological properties presented by cinnamon, such as antibacterial, antifungal and antioxidant properties, recent studies highlighted its action on postprandial blood glucose, triacylglycerol, and total and LDL-cholesterol concentrations, both in healthy and type 2 diabetes patients. Inclusion of cinnamon in the diet, in doses varying from 3g to 6g, seems to be beneficial for controlling glucose metabolism in nondiabetic adults during postprandial period^{1,2}. However, once botanical species of cinnamon from different countries may present high levels of toxic elements, such large daily intakes of cinnamon can easily exceed their maximum permissible limits and, consequently, promoting adverse health effects. This study was designed to investigate the elemental profile of cinnamon powder samples (branded and bulk) available in the Portuguese market and to assess the cumulative non-carcinogenic risk to human health due to the exposure to mixtures of such elements through the consumption of 6 g of cinnamon in the diet. Branded and bulk cinnamon samples were randomly purchased and elemental concentrations determined by wavelength dispersive X-ray fluorescence spectroscopy (WDXRF). In each sample, elemental concentrations were estimated using standardless and specific calibration WDXRF methods. Although the concentration of each element varied among brands and/or bulk cinnamon samples, WDXRF analysis revealed a common elemental pattern among all samples tested: the presence of approximately 16 elements, such as Ca, K, S, P, Si, Mg, Fe, Mn, Mo, Cl, Sr, Cu, Zn, Ru, Al, and Br. The non-carcinogenic risk was calculated according to EPA guidelines through the Hazard Quotient (HQ) for each element³. Even though the HQ obtained was lower than the USEPA guideline of 1, the combined non-carcinogenic effects of all toxic elements, expressed as Hazard Index (HI), revealed a potential human health risk for heavy consumers of cinnamon (HI= 1.18)⁴.

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Keywords: Chemical contaminants and risk assessment

**Prevalence of DON, 3-ADON and 15-ADON in spring wheat grain from different agricultural production systems in Lithuania**

Audronė Mankevičienė¹; Sigita Janaviciene¹; Yuliia Kochiiuru¹

ABSTRACT

Deoxynivalenol (DON) together with two acetylated derivatives, 3-ADON and 15-ADON is present in cereal grains and their products. 15-ADON is more toxic than DON and 3-ADON. Co-occurrence of DON and acetylated derivatives in cereal grain is found worldwide. Until now, DON and its derivatives have been considered equally toxic by health authorities. In this survey, 103 spring wheat samples were analysed for co-occurrence of type-B trichothecenes (DON, 3-ADON, 15-ADON) from different agricultural production systems in Lithuanian. Samples were classified according to the production systems – organic, conventional and intensive. Mycotoxin levels in the spring wheat grain samples were determined by the HPLC method with UV detection. Results showed that a type-B trichothecenes tended to be present at higher concentrations in the grain from the intensive production system. Eighty one percent of the spring wheat grain samples from the intensive production system were co-contaminated with a combination of DON+3-ADON+15-ADON, 1% with DON+3-ADON and 3-ADON. DON+15-ADON and DON were found in 5% and 10% of the tested samples respectively. Two percent of samples were free from mycotoxins. In the grain samples from the conventional production system, DON and combination of DON+3-ADON showed a higher incidence - 47% and 23%, respectively. The samples with a combination of DON+3-ADON+15-ADON accounted for 18%. Completely different results were obtained from the analyses of organic grain samples. A great number of organic spring wheat grain samples were contaminated with DON+3-ADON (55%), DON (36%). The combinations of DON+3-ADON+15-ADON and DON+15-ADON were present at the lowest concentrations - 0% and 9%, respectively. The production systems did not lead to significant differences in mycotoxin levels, although a trend toward higher incidence and higher contamination was observed in the samples from intensive and conventional production systems.

Keywords: Trichothecenes, spring wheat, co-occurrence, grain production systems

β -1,3-glucanase activity by *Pichia* sp. as biocontrol mechanism against toxigenic *Aspergillus* sp.

Mariana Lino De Souza ¹; Gustavo Magno Dos Reis Ferreira ¹;
Luís Batista ¹; Cristina Ferreira Silva ¹

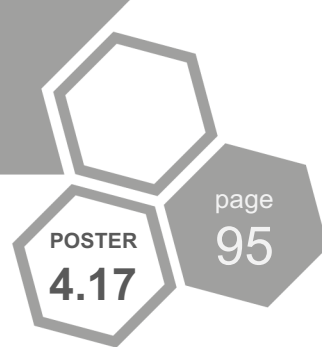
ABSTRACT

Yeasts are important microorganisms in different biotechnological processes, including the prevention of toxigenic fungi contamination in food during the food processing especially agricultural products. In this way, there is a reduction in the application of fungicides, making the production process safer for human consumption. Biocontrol can occur through different mechanisms including the production of enzymes, like β -1,3-glucanase that has the ability of degrade the cell wall of fungi, once the cell wall of the fungi its compost by chitin and β -glucans. The objective of this study was to evaluate the activity of the enzyme β -1,3-glucanase, produced by yeasts of the genus *Pichia*, using as a carbon source cell wall preparation (PPC) of *Aspergillus carbonarius* and *A. ochraceus*. The isolates tested have already been characterized as antagonists of *Aspergillus* sp. The enzymatic activity was quantified by the PAHBAH method. Cultivation was carried out for 7 days at 28 ° C under stirring. *P. anomala* presented higher activity in the prepared medium containing PPC of *A. carbonarius* (0.325 μ g glucose/min.) and *P. holstii* in the medium containing *A. ochraceus* PPC (0.263 μ g glucose/min). The enzymatic activity was low indicating that the antagonistic activity of the tested isolates was not exclusively due to degradation of the cell wall of the filamentous fungi and therefore more than one mechanism is involved in the biocontrol of toxigenic fungi.

Financial Support: CAPES, FAPEMIG, CNPq

Keywords: Biocontrol, yeast, toxigenic fungi

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Use of wild yeasts as a biocontrol agent against toxigenic fungi and OTA production

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Rosane Schwan ⁵; Cristina Silva ¹

ABSTRACT

This study evaluated the antagonistic potential of 32 wild yeast isolates from coffee and cocoa bean fermentation. These yeasts were inoculated in co-cultivation with *Aspergillus carbonarius* (CCDCA 10608 and CCDCA 10408) and *Aspergillus ochraceus* (CCDCA 10612) isolated from grapes and coffee beans. The mycelial growth and ochratoxin A (OTA) production were evaluated, and the spores were counted after cultivation at 28°C for seven days. The yeasts presented higher inhibitory effects (53% in relation to the control) over the mycelial growth of the isolated *A. ochraceus* (CCDCA10612). *Pichia anomala* CCMA0148 and *Saccharomyces cerevisiae* CCMA0159 provided the greatest inhibition of the growth of all fungal strains. All *Pichia* species presented the highest inhibitory effects on the production of spores, and *S. cerevisiae* CCMA 0159 at concentrations of both 104 and 107 mL⁻¹ cells inhibited the production of spores by 100%. *Rhodotorula mucilaginosa* was effective at inhibiting OTA production by the three isolates of *Aspergillus*. *S. cerevisiae* CCMA0159 and *Pichia anomala* CCMA0148 showed high potential as biocontrol agents in the conditions tested.

Financial Support: CNPq, CAPES and FAPEMIG.

Keywords: Antagonistic activity, ochratoxin A, *Aspergillus*, inhibition growth, *Pichia sp.*, *Saccharomyces sp.*

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Gamma irradiation in ochratoxin A control

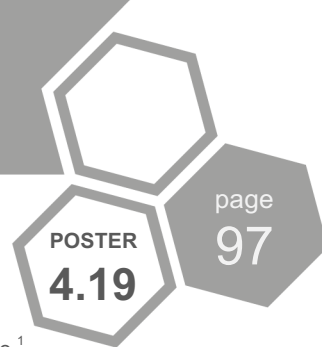
Thalita Calado¹; Cabo Verde²; Armando Venâncio¹; Luís Abrunhosa¹

ABSTRACT

Ochratoxin A (OTA) is one of the main mycotoxins found in food. Gamma radiation is used for preserving foods since it inactivates pathogens. The effect of irradiation on mycotoxins has been studied but results are contradictory. The different matrices and conditions used by several authors may have influenced reported results. The aim of this work was to study the effects of gamma radiation in OTA in order to evaluate its applicability in foods. OTA was irradiated in its dry form, in aqueous and in methanolic solutions to evaluate the water role in OTA irradiation. Then, OTA contaminated food matrices, such as wheat flour, grape juice and wine, were irradiated. In aqueous solutions, high degradation of OTA ($\geq 90\%$) was obtained with gamma radiation doses as low as 2.5kGy. However, in dried samples, OTA was found extremely resistant to radiation doses of up to 10kGy. In this case, water was a determinant factor for the effectiveness of irradiation process. In the assays with food matrices, radiation doses greater than 10kGy were needed to achieve higher reductions of OTA, being eliminated just 24%. It was also observed that OTA elimination was higher in wheat flour with higher moisture contents but the elimination of OTA in grape juice and wine were not higher than those observed in wheat flour. It is concluded that OTA is very sensitive to irradiation in water solutions but resistant in its dry form and in food matrices. Due to the low elimination percentage observed, it can be considered that gamma radiation is not a suitable technology for the elimination of OTA from foods.

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Keywords: Ochratoxin A, gamma irradiation, grape juice, wheat flour, wine



Flow cytometry as a tool for assessing spore cell viability

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ABSTRACT

The presence of filamentous fungi can be a problem due to their capacity to produce mycotoxins. Several methods to inactivate spores have been studied; however, the evaluation of spores' viability is time consuming. Flow cytometry (FC) is a method to evaluated quickly and simultaneously several characteristics of cells, including its viability. To evaluate the efficacy of heat, gamma radiation and ozone in inactivating *Aspergillus parasiticus* spores, FC together with propidium iodide (PI) staining was used. Gamma irradiation was performed at doses between 0.5 and 10 kGy. Ozone treatment was done with aqueous ozone (10 or 20 mg/L) for 0 to 60 min exposure. The heat treatment (autoclaved by 20 min at 121 °C) was used as a control. FC was performed in a Sony EC800 and the method was adapted from Mesquita *et al.* (2013). Briefly, 40 µL of sample (untreated or treated with different sterilization methods) was added to 360 µL of PI solution (25 µg/mL) and incubated for 10 min in the dark. Thereafter, samples were mixed and analysed by flow cytometry. FC together with PI was effective to assess cellular viability of spores treated with heat and ozone solution. The inactivation of *A. parasiticus* was effective with ozone with short exposure time. The best results were obtained with ozone at 10 mg/L where an inactivation efficiency of 97% was observed. With gamma radiation, FC with PI florescence was not effective. Probably because with irradiation the cellular membrane is not degraded but the DNA is. Thus, PI cannot enter the cell and bind to the DNA chain explaining the absence of fluorescence.

Reference: Mesquita, N., et al. International Biodeterioration & Biodegradation 84 (2013): 250-257.

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Keywords: Flow cytometry, spore viability, gamma radiation, ozone

Gaseous ozonation to reduce aflatoxins levels and microbial contamination in corn grits

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ABSTRACT

Corn is one of the most cultivated cereals in Brazil. Their grains are constantly exposed to contamination by mycotoxins which are secondary metabolites produced by fungi. The adoption of quality management systems during the maize production chain is essential to ensure food safety in terms of mycotoxin contamination. *Canjiquinha*, a product from corn grits, is a cultural food easily purchased by the Brazilian consumer at low prices. Corn grits is a raw material used to produce a large variety of corn products by the food industry. Some studies have demonstrated a high contamination of this product by aflatoxins representing a potential risk of exposure due to its contamination. In this work, it was evaluated the efficacy of gaseous ozonation on the levels of aflatoxins B₁, B₂, G₁, G₂ and on the microbial contamination in corn grits. The application of gaseous ozone was tested in different combinations of exposure time, ozone concentration and *canjiquinha* mass, being these independent variables investigated. After the ozonation treatment samples were collected to aflatoxins and microbiological analyses. Aflatoxins, by HPLC-FD and pre-column derivatization and microbiological to analyze aflatoxins producers fungi and mesophilic bacteria. Results show reductions up to 57% in aflatoxins levels. Total fungal count reduced around 3.0 cycles log CFU/g and total mesophilic count were reduced until non detectable levels. Results demonstrated that ozonation is an effective alternative to reduce aflatoxin and microbial contamination in corn grits and, consequently, improving the safety of this food.

Keywords: Mycotoxins, ozone, zea mays

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Acrylamide mitigation in bakery products

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ABSTRACT

The aim of this study was the determination of acrylamide in Portuguese bakery and its reduction in a bakery product. Acrylamide is considered by the International Agency for Research on Cancer as a carcinogenic compound to animals and probably to humans. For this study a total of 30 samples of god bread, “trouxa filó”, pies, ham and cheese rollings, muffins, pastels, and cookies were randomly collected in several commercial establishments. Sample preparation involved solid phase extraction and for the quantification of acrylamide a ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) was used. The ham and cheese rolling and “trouxa filó” had the highest amount of acrylamide, 3743 µg/kg, and 3862 µg/kg, respectively. The results also showed that caramel cookies, butter cookies, Greek cookies and cocoa cookies do not exceed the EFSA indicative value (500 µg/kg) (EFSA, 2015). Pie samples(686-1084 µg/kg), god's bread(995 µg/kg), pastels(527-809 µg/kg) and muffins(676-1057µg/kg) contain high levels of acrylamide when compared to the values found in the literature for bakery products, 198 µg/kg (Mojska, Gielecińska, Szponar, & Ołtarzewski, 2010). Given the obtained results, tests were carried out in order to reduce the concentration of acrylamide. A bakery product was prepared to which four different reducing agents (A, B, C and D) were individually added. The effect of each agent on acrylamide formation was evaluated. Results showed that mixture B obtained an acrylamide reduction of 5.6%. On the other hand, the remaining mixtures increased the production of the contaminant. Yet, it was found that the decrease obtained with mixture B is still not sufficient since it remains above the indicative value of EFSA. Thus, further studies are necessary in order to achieve a higher percentage of reduction of acrylamide. Progress studies are ongoing with other reducing agents and flours.

Keywords: Acrylamide, occurrence, mitigation

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Application of N2a assay on the analysis of emerging marine biotoxins and further confirmation by LC-MS/MS

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ABSTRACT

Marine biotoxins are natural contaminants of the marine environment produced as secondary metabolites by bacteria and microalgae. Several conditions affecting the marine environment and in particular climate change seem to be the responsible for the emergence of new or already existing marine biotoxins in different geographical areas. Ciguatoxins (CTX) and Tetrodotoxins (TTX) can be a very good example of emerging toxins in areas where they have never been previously reported. These toxins appeared in European waters, associated to several fish species from Canary Islands and Madeira and also in imported fish from Pacific and Indian Oceans, while in the case of TTX, which is a toxin commonly found in tropical regions and associated to fish contaminations, although this toxin have been recently found in filter-feeding mollusks in several locations of Europe. Both toxins pose a potential threat to human health and therefore need to be properly identified and confirmed. Several analytical methods have been developed worldwide. Screening methods such as Neuroblastoma 2a assay (N2a) have been developed to identify the toxicity of samples contaminated, while instrumental methods such as Liquid Chromatography coupled to tandem Mass Spectrometry (LC-MS/MS) has been developed for their confirmation. N2a is being used as a powerful sensitive tool for toxicity evaluation. The lack of standards and reference materials, in particular for CTXs, has been considered the main limitation to advance on the optimization of the LC-MS/MS analysis of CTXs and TTX. The complexity of the matrix is also an important analytical challenge, being the optimization of the sample pretreatment a key issue that still needs to be resolved. N2a has been used to a first toxicity screen of the samples and also as a tool to confirm the efficiency of the different steps included in the analytical protocol for LC-MS/MS analysis as well as for confirmation purposes.

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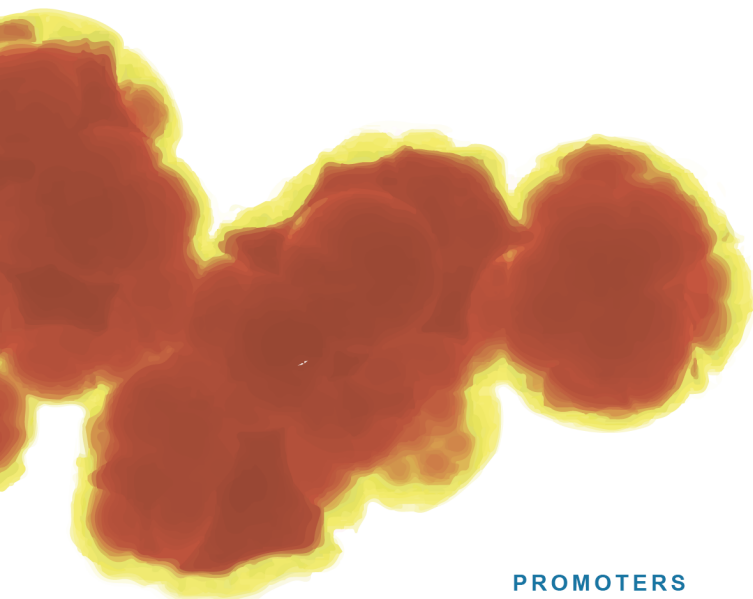
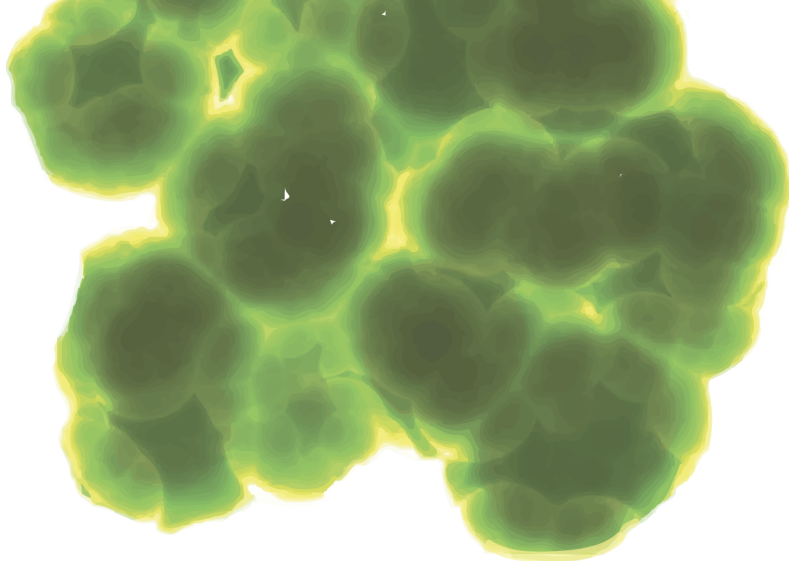


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