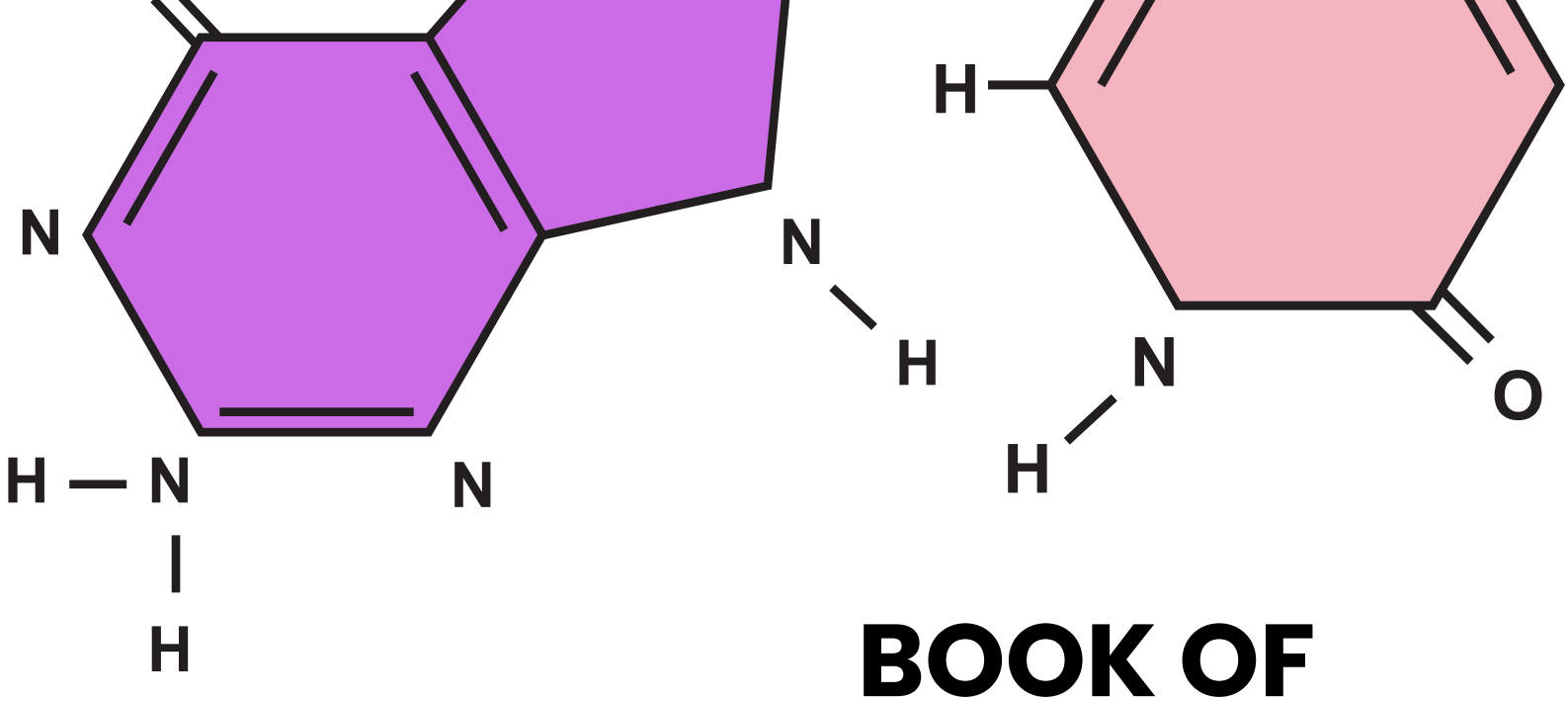
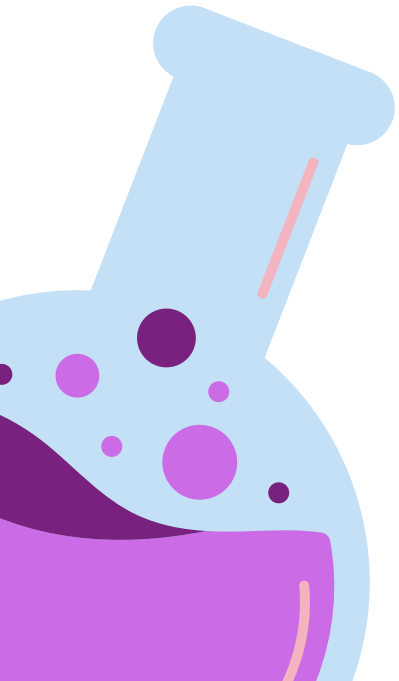


BOOK OF ABSTRACTS

NETWORKING

FOOD SAFETY TOOLS FOR MYCOTOXINS AND ACRYLAMIDE RISK EXPOSURE ASSESSMENT

RISK TOX



University of Valencia
Research group (GIUV2021-513)

GENERALITAT VALENCIANA
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VNIVERSITAT DE VALÈNCIA Faculty of Pharmacy and Food Sciences

RiskTox reserach group of University of Valencia (GIUV2021-513) gratefully acknowledge the financial support of the *Conselleria d´Educació, Universitats i Ocupació, Generalitat Valenciana* (CIAORG/2024/063) for the organization of this 4th Workshop. We also thank the Spanish Ministry of Science, Innovation and Universities (MICIU / AEI / 10.13039/501100011033 / FEDER, EU; PID2024-160527OB-I00) for its support in the realization of this scientific meeting.

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Editado por: Dr. Yelko Rodríguez-Carrasco

RiskTox – Grupo de investigación de la Universitat deValència (GIUV2021-513)

Coordinado por: Dra. Houda Berrada, Dr. Yelko Rodríguez-Carrasco

TABLE OF CONTENTS

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• Dra. Houda Berrada Ramdani	Universitat de València
• Dra. María José Ruiz Leal	Universitat de València
• Dra. Mónica Fernández Franzón	Universitat de València
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SCIENTIFIC PROGRAMME

Workshop: NETWORKING ON FOOD SAFETY TOOLS FOR MYCOTOXINS AND ACRYLAMIDE RISK EXPOSURE ASSESSMENT

DATE: Friday, November 21st, 2025

VENUE: Faculty of Pharmacy and Food Sciences, Burjassot, Valencia, Spain

LUNCHEON: 13:00-14:30 (Bar of the Faculty).

Workshop: 09:30 to 17:00 (Saló d'Actes)

09:30-10:00 Registration and welcome coffee

10:00-10:15 Institutional welcome and Opening

10:10-10:40 ***Plenary session 1***
Chairpersons: Prof. Josefa Tolosa, Prof. Yelko Rodríguez

10:10-10:40 *Opening conference*

Title: "Integrating targeted and non-targeted mass spectrometry in human biomonitoring of mycotoxin"

Dr. Yelko Rodríguez-Carrasco*, Universitat de València (Spain)*

10:40-12:00 *Oral sessions*

10:40-11:00 Title: "Mapping mycotoxins and acrylamide: Tools for safer food and risk assessment"

Dr. Luana Izzo, Università degli Studi di Napoli "Federico II" (Naples, Italy)

11:00-11:20 Title: "Food safety in South Africa: Can nixtamalization of maize reduce mycotoxin exposure?"

Dr. Elias Maris, Centre of Excellence in Mycotoxicology and Public Health, Department of Bioanalysis (Ghent, Belgium)

11:20-11:40 Title: "Tracking mycotoxin exposure in Portugal: New insights and key determinants"

Dr. Paula Alvito, Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Lisbon (Portugal)

SCIENTIFIC PROGRAMME

11:40-12:00 Title: “How much acrylamide are we really eating? A scientific look at dietary exposure and bioaccessibility”

Dr. Marta Mesias, Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid (Spain)

12:00-13:00 Posters viewing

13:00-14:30 Invited members Luncheon

14:30-16:20 ***Plenary session 2***
Chairpersons: Prof. Mónica Fernández, Prof. Mercedes Taroncher

14:30-15:50 *Oral sessions*

14:30-14:50 Title: “Alternative models for high-throughput toxicity screening: Intestinal and hepatic organoids derived from mouse tissues”

Dr. Maria Cristina Gonzalez-Montero, Universidad de León (Spain)

14:50-15:10 Title: “The enteric nervous system: A gateway for foodborne neurotoxicity”

Dr. Miguel Faria, LAQV-REQUIMTE, Universidade do Porto (Porto, Portugal)

15:10-15:30 Title: “Impact of choronic low dose exposure to a mixure of emerging contaminants on the inflammatory status promoting the onset of non-communicable diseases, such as Inflammatory Bowel Diseasm (IBD)”

Dr. Maria Grazia Zizzo, Università degli Studi di Palermo (Palermo, Italy)

15:10-15:30 Title: “A two-compartment microfluidic device with integrated barrier function analysis for investigating epithelial-immune cell interactions in the gut”

Dr. Jose M. de Hoyos-Vega, Mayo Clinic, Rochester, USA

SCIENTIFIC PROGRAMME

15:50-16:20 *Closing conference*

Title: “Food toxicity assessment in the 21st Century: How far are we from alternatives?”

***Dr. Veronica Zingales**, Universitat de València (Spain)*

16:20-17:00 Roundtable
Chairpersons: Prof. Houda Berrada, Prof. María José Ruiz

17:00 Closing

20:30 Workshop dinner/Social event

INDEX OF ABSTRACTS

ORAL COMMUNICATIONS

INDEX OF ABSTRACTS

POSTER COMMUNICATIONS

ORAL COMMUNICATIONS

Integrating targeted and non-targeted mass spectrometry in human biomonitoring of mycotoxin

Rodríguez-Carrasco, Yelko; Fernández-Franzón, Mónica; Moltó, Juan Carlos; Berrada Houda

*Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estelles s/n, 46100 Burjassot, Valencia, Spain. *E-mail: Yelko.Rodriguez@uv.es*

Abstract

Human biomonitoring (HBM) of mycotoxins is a key approach for assessing internal exposure to these widespread foodborne contaminants and their metabolites. Targeted liquid chromatography–tandem mass spectrometry (LC–MS/MS) remains the benchmark for sensitive and accurate quantification of mycotoxins. Complementary non-targeted high-resolution mass spectrometry (HRMS) enables discovery-driven screening, retrospective data analysis, and comprehensive profiling of exposure biomarkers. This work discusses recent advances and practical frameworks for integrating targeted and non-targeted MS within HBM studies. The combined strategy merges the quantitative precision of targeted LC–MS/MS with the exploratory capacity of HRMS to capture both known and previously unrecognized biomarkers. Emphasis is placed on harmonized sample preparation, data-independent acquisition (DIA) workflows, and computational tools for spectral deconvolution, annotation confidence, and semi-quantification of new features. Case studies from European biomonitoring programs demonstrate how this integrated approach has uncovered complex co-exposure patterns, novel phase I and II metabolites, and biomarker candidates with potential toxicological relevance. Data integration using suspect screening lists, *in silico* fragmentation, and machine learning-based annotation further enhances identification reliability and throughput. Bridging targeted quantification and non-targeted discovery expands analytical depth and interpretability in mycotoxin exposure assessment. This comprehensive framework strengthens the scientific basis for refined risk evaluation, exposure mitigation, and regulatory decisions, contributing to a deeper understanding of the human mycotoxin exposome within the One Health perspective.

Keywords: Human biomonitoring; mycotoxins; target and non-targeted mass spectrometry; exposure assessment

Acknowledgements: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00.

Mapping mycotoxins and acrylamide: Tools for safer food and risk assessment

Navarré, A.[1,2] ; Ledenko, I.[1] ; Manzi, B.[1] ; Di Matteo, A.[1] ; Rodríguez-Carrasco, Yelko [2]; Izzo, Luana [1]

[1]FoodLab, Università degli Studi di Napoli "Federico II", Italy; [2]Laboratory of Food Chemistry and Toxicology, Universitat de València, Spain

Abstract

Food contaminants are substances that may be unintentionally present in food products and can pose risks to human health. Among these, process contaminants such as acrylamide (AA) and natural contaminants such as mycotoxins are of particular concern due to their toxicological relevance and widespread occurrence. Considering that Italy is among the European countries with the highest consumption of biscuits, a total of 34 commercial samples, were analyzed using QuEChERS extraction and UHPLC-Q-Orbitrap HRMS determination to detect the presence of AA and mycotoxins (trichothecenes, enniatins, aflatoxins, zearalenone, and beauvericin). In turn, using the latest available data on food consumption and anthropometric measurements of the Italian population (INRAN SCAI IV study), a risk assessment was carried out. Results showed that all samples were contaminated with AA, with values ranging from <LOQ (0.195 µg/kg) to 167.7 µg/kg, with an average level of 79.7 µg/kg. Likewise, the co-occurrence of several mycotoxins was observed across the samples. At least one enniatin (A, A1, B, or B1) was detected in nearly all samples ranging from <LOQ to 100.8 µg/kg. DON occurred in 68% of the samples (from <LOQ to 101.5 µg/kg) and ZEA in two samples, at concentrations of 4.98 and 6.13 µg/kg, respectively. No sample exceeded the maximum limits established by EC Regulations and benchmark levels for AA. Regarding risk assessment, a potential carcinogenic hazard was observed for the median amount of AA detected in all ranges of the male and female population between 1 and 17 years of age, with MoE values <10,000, while no sample exceeded 5% of the TDI with the average values found for DON and ZEA (TDI: 1 and 0.25 µg/kg bw/day, respectively). The results highlight the importance of continuous food monitoring to maintain food safety, as well as constant review of legislation to implement improvements in contaminant reduction.

Keywords: Biscuits, acrylamide, mycotoxins, risk assessment, food safety.

Acknowledgments: Project "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods" funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for proposals No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU Project code PE00000003, CUP D93C22000890001. This research was also funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00. A. Navarré is thankful for the ACIF grant (CIACIF/2024/259) provided by the Generalitat Valenciana.

Food Safety in South Africa: Can Nixtamalization of Maize Reduce Mycotoxin Exposure?

Maris, Elias[1, 2]; Ndlangamandla, Palesa [3]; Abayomi Adelusi, Oluwasola[1]; Festus Akinmoladun, Oluwakamisi[3]; Olayemi Odukoya, Julianah[1, 3]; Temitope Fagbohun, Richard[3]; Adeoye Oyeyinka, Samson[3,4]; Sekhejane, Palesa[3]; Pero-Gascon, Roger[1, 5]; De Boevre, Marthe[1]; Croubels, Siska[6]; Berka Njobeh, Patrick[3]; De Saeger, Sarah[1, 3]

[1]Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Belgium; [2]Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Belgium; [3]Department of Biotechnology and Food Technology, Faculty of Science, Doornfontein Campus, University of Johannesburg, South Africa; [4]School of Agr-Food Technology and Manufacturing, College of Health and Science, University of Lincoln, United Kingdom; [5]Department of Chemical Engineering and Analytical Chemistry, Institute for Research on Nutrition and Food Safety (INSA-UB), University of Barcelona, Spain; [6]Laboratory of Pharmacology and Toxicology, Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium

Abstract

Mycotoxin contamination is a global threat to food safety and human health, particularly in regions with high food insecurity. This study evaluates the effectiveness of nixtamalization, a traditional food processing method, in reducing mycotoxin levels in raw maize and their corresponding urinary biomarkers of exposure. Forty participants from Kliptown, Soweto (South Africa), were randomly assigned to consume either control maize, or visibly moldy maize subjected to nixtamalization. First-morning urine samples were collected on day 1, 3, and 5 to assess exposure to multiple mycotoxins (40) using a validated LC-MS/MS method. Participants received two portions of either control or nixtamalized maize, prepared as a porridge, on the 2nd and 4th day of the study. Nixtamalization achieved a complete reduction of FB3 and DON to <LOD levels in maize, while reducing FB1, FB2, and ZEN by 95.1%, 94.8%, and 89.0%, respectively. AFB1 was <LOQ in moldy maize and undetectable after processing. Biomarker analysis revealed that after consumption urinary levels of FB1, ZEN, and its metabolites α- and β-ZOL did not show significant differences between both groups. These results indicate that consumption of nixtamalized maize led to internal exposure levels comparable to those from the control maize. DON and tenuazonic acid levels were not affected by the intervention ($p>0.05$), with urinary detection frequencies remaining above 90%. This study highlights nixtamalization as an effective strategy for reducing mycotoxin levels in maize and presents its benefit in lowering internal mycotoxin exposure.

Keywords: Mycotoxin, Maize, Nixtamalization, Sub-Saharan Africa, Human Biomonitoring, Food safety

Acknowledgments: This research was conducted within the framework of the ERA-NET LEAP-Agri MYCO-SAFE-SOUTH project, and MYTOX-SOUTH project, with financial support from the South African National Research Foundation (NRF) and The World Academy of Sciences (TWAS). Additional funding was provided by FoodBev SETA, and the Maize Trust.

Tracking mycotoxin exposure in Portugal: New insights and key determinants

Namorado, S[1,2]; Maris, E[3,4]; Chen, A[3]; Pero-Gascon, R[3,5], De Boevre, M[3], De Saeger, S[3]; Silva, MJ[1,2]; Alvito, P[1,6]

[1]National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal. [2]Comprehensive Health Research Center (CHRC), NOVA University of Lisbon, Lisbon, Portugal. [3]Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharmaceutical Sciences, Ghent University, Belgium. [4]Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium. [5]Department of Chemical Engineering and Analytical Chemistry, Institute for Research on Nutrition and Food Safety (INSA-UB), University of Barcelona, Spain. [6]Centre for Environmental and Marine Studies (CESAM), University of Aveiro, Aveiro, Portugal.

Abstract

Human biomonitoring (HBM) is a vital tool for assessing exposure to environmental chemicals. Mycotoxins have been associated with various adverse health effects, including estrogenic, immunotoxic, nephrotoxic, and teratogenic outcomes. In this presentation an overview of two recent HBM studies on multiple mycotoxins exposure biomarkers conducted in Portugal will be given. The first study analyzed 37 mycotoxin biomarkers in urine samples from 94 adult participants (48.4 ± 15.2 years), as part of the National Food, Nutrition, and Physical Activity Survey (2015–2016). Six different mycotoxins -DON, ZEN, AOH, OTA, FB1, and CIT- was confirmed through the quantification of 12 urinary biomarkers in paired 24 h and first-morning urine samples. DON and its metabolites were among the most frequently detected biomarkers and AOH was identified for the first time in urine samples from a European population. Associations between urinary mycotoxin biomarkers and consumption of specific food items were also observed. More recently, a subset of 295 first-morning urine samples from adults (28–39 years) was collected between 2019-2020, as part of a cross-sectional study embedded within the Portuguese National Health Examination Survey (INSEF). These samples were analyzed using a newly optimized and validated LC-MS/MS method capable of detecting 40 mycotoxins and/or their metabolites in urine. DON and tenuazonic acid were the most frequently detected, with detection rates of 85% and 96%, respectively. Further investigations into key exposure determinants—including dietary habits, demographic factors, and geographical variations—are ongoing and will be presented. Altogether, these findings highlight the importance of continued surveillance and the integration of HBM into national food safety and public health strategies.

Keywords: Human biomonitoring; mycotoxins; urinary biomarkers; *Food safety*

Acknowledgements: The INSEF-ExpoQuim study is supported under the HBM4EU project co-funded by Horizon 2020 (No. 733032). Thanks also to CESAM, ToxOmics and CHRC by FCT/MCTES through national funds (UIDP/50017/2020, UID/50006/2020+LA/P/0094/2020(doi.org/10.54499/LA/P/0094/2020), UIDP/00009/2020, UIDB/00009/2020, UIDP/04923/2020, UIDB/04923/2020). E.M is supported by an iBOF project (No. 01IB1320); A. Chen by an FWO PhD fellowship (11A0725N); R. P-G by an FWO postdoctoral fellowship (12D4423N) and M. De Boevre by the European Research Council (No. 946192, HUMYCO).

How much acrylamide are we really eating? A scientific look at dietary exposure and bioaccessibility

Mesias, Marta

Institute of Food Science, Technology and Nutrition (ICTAN-CSIC)

Abstract

Since acrylamide was discovered in processed foods, numerous measures have been taken in the EU to monitor and reduce its presence. EFSA launched a monitoring database in 2007, and the European Commission introduced indicative values in 2011, updated in 2013, to include thresholds for coffee, cereal-based products, and processed potatoes. These values were further revised in 2017 with the publication of Regulation (EU) 2017/2158, which established benchmark levels and mitigation strategies for food business operators. EFSA's 2015 opinion estimated average dietary acrylamide exposure to range between 0.4 and 1.9 μg/kg bw/day, raising concerns about potential neoplastic risks. Consequently, controlling acrylamide levels in processed foods remains a key public health priority. Most studies evaluating dietary acrylamide exposure have focused on industrially produced foods, often overlooking acrylamide levels in foods prepared in other settings. Recent studies conducted in catering environments, such as school, university, workplace, and senior center cafeterias, have reported that acrylamide exposure in all population groups may be associated with an increased risk of neoplastic effects. These findings underscore the importance of implementing mitigation strategies during food preparation in collective catering and promoting appropriate consumer education on food safety to encourage safer and healthier choices. It is also important to consider the acrylamide bioaccessibility during digestion, since it must be absorbed to exert toxic effects. Although traditionally assumed to be fully bioavailable, digestive processes may affect acrylamide stability. Due to its reactivity with nucleophilic groups, acrylamide may bind to proteins, limiting its availability. Recent studies show acrylamide levels may decrease during digestion, particularly in protein-rich matrices, supporting the need to reassess exposure estimates. Understanding acrylamide's bioaccessibility is critical for refining risk assessments and regulatory decisions. Blocking its absorption through interactions with dietary components may help mitigate toxic effects, and future EU policies should account for digestive transformations when evaluating acrylamide risks.

Keywords: Acrylamide, Exposure, Bioaccessibility, Risk, Food safety

Acknowledgments: This research was funded by project ACRYREVAL (PID2022-137697NB-I00), funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe".

Alternative Models for High-Throughput Toxicity Screening: Intestinal and Hepatic Organoids Derived from Mouse Tissues

González-Montero, María-Cristina[1]; Galli, Giulio[1]; García-Estrada, Carlos[1,2]; Balaña-Fouce, Rafael[1,2]

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Abstract

Traditional toxicological assessments often rely on *in vivo* models or immortalized cell lines, which despite their utility, present significant limitations in terms of scalability, physiological relevance and ethical concerns. Three-dimensional organoids systems have emerged as promising tools that better reflect tissue architecture and function while supporting the development of alternative testing strategies. In this work, we present intestinal and hepatic organoids derived from mouse tissues as physiologically relevant and experimentally tractable models for toxicology applications. These organoids retain key features of their tissue of origin, including structural organization, cell-type diversity and functional activity, making them suitable for evaluating tissue-specific responses *in vitro*. To facilitate high-throughput screening, we have developed and optimized protocols for generating, culturing and assaying these organoids in 384-well plate formats. This miniaturized system supports automated handling, reduced reagent use and scalable screening workflows. Additionally, preliminary co-culture strategies have been explored by incorporating bone marrow-derived macrophages into hepatic organoid system. This aims to enhance the physiological relevance of the model by enabling the study of immune-mediated mechanisms and tissue-immune interactions. Derived from murine tissues, these 3D systems offer a practical intermediate model that combines biological complexity, bridging the gap between conventional *in vitro* systems and *in vivo* studies. Their implementation in high-throughput-compatible platforms holds promise for advancing alternative toxicological testing.

Keywords: intestinal and hepatic organoids, toxicology, high-throughput screening, co-culture.

The Enteric Nervous System: A Gateway for Foodborne Neurotoxicity

Faria, Miguel A.; Ramos, Helena; Araújo, A. Margarida; Ferreira, Isabel MPLVO

LAQV-REQUIMTE, Faculty of Pharmacy, University of Porto, Rua Jorge de Viterbo Ferreira, 228, 4050-313, Porto, Portugal

Abstract

The enteric nervous system (ENS) is a vast neuronal network within the gastrointestinal (GI) tract, interspersed with enteric glial cells. It plays essential roles in maintaining intestinal homeostasis, supporting neuronal function, and modulating neuroimmune interactions. As a key component of the gut–brain axis (GBA), the ENS has been increasingly implicated in the pathogenesis of neurodegenerative diseases (NDs). Because the GI tract is the primary interface with diet, the ENS is also the first neural structure exposed to food chemical contaminants (FCCs). Emerging evidence suggests that FCCs may initiate or amplify neurotoxic processes; however, their specific effects on the ENS and its role in mediating central neurotoxicity remain poorly understood. This presentation synthesizes current evidence on FCC-induced alterations in the ENS and examines its involvement in gut-to-brain neurotoxicity. Reported FCCs include acrylamide, mycotoxins, micro/nanoplastics, and bisphenols, primarily investigated using *in vivo* models. FCC exposure consistently induces neurochemical remodeling of the ENS and activation of enteric glia, often accompanied by inflammation, histological changes, and GI dysfunction. Notably, compounds such as rotenone, paraquat, and polystyrene micro/nanoplastics promote α-synuclein aggregation within the ENS and its vagal propagation to the brain. Vagotomy-based models further demonstrate that disrupting ENS–CNS communication attenuates FCC-related central neurotoxicity, supporting the hypothesis that the ENS mediates gut-to-brain propagation of neurotoxic signals. These findings position the ENS as a critical, yet underexplored, interface in food exposome-related neurotoxicology. Future research should prioritize long-term, low-dose, and mixture exposure studies that better reflect realistic dietary intake, as well as advanced *in vitro* models for mechanistic interrogation of ENS-mediated signaling to the brain.

Keywords: Enteroglia; Food Contaminants; Neurodegenerative Diseases; Gut-Bran Axis

Acknowledgments: Miguel A. Faria acknowledges FCT the researcher contract through CEECINSTLA/00029/2022. Helena Ramos thanks FCT for her Ph.D. grant (2024.01455.BD). This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Impact of choronic low dose exposure to a mixure of emerging contaminants on the inflammatory status promoting the onset of non-communicable diseases, such as Inflammatory Bowel Diseasm (IBD)

Zizzo, Maria Grazia

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Abstract

Humans are continuously exposed to a wide range of environmental pollutants, including a broad spectrum of organic and inorganic compounds. Most of the in vitro and in vivo studies investigated the effects on single compounds, while real-life exposure concerns a complex mixture of environmental chemicals, often at low or subtoxic concentrations, encountered daily and throughout our lives. Growing evidence suggests that such mixtures, even in the absence of overall cyto- and genotoxicity, can present potential health implications that may contribute to the rising global prevalence of Non-Communicable Diseases (NCDs), as Inflammatory Bowel Diseases (IBDs). In this study, using an animal model of chemical induced colitis, we aimed to examine the effects of prolonged, low-dose exposure to two contaminants frequently detected in the Sicilian environment: 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), a commonly used flame retardant, and Estrone (E1), a naturally occurring estrogen to elucidate the contribute in gut dysregulation and the pathogenesis of IBDs. Wistar rats were orally exposed to two different low-dose mixtures of BDE-47 and E1 for 30 or 60 days prior to colitis induction via intrarectal 2',4'-dinitrobenzenesulfonic acid (DNBS). Intestinal tissues from untreated and treated sham and DNBS-exposed animals were analyzed to assess inflammation, intestinal damage. The exposure to pollutants alone did not cause evident alterations in gut morphology or function, however it significantly increased susceptibility to DNBS-induced colitis. After 30 days of exposure, rats exhibited greater weight loss, reduced food intake, and elevated myeloperoxidase (MPO) activity compared to the DNBS-only group. These effects were further exacerbated after 60 days, with animals displaying more severe intestinal damage. These preliminary findings suggest that chronic exposure to low doses of environmental contaminants may compromise gut health by exacerbating intestinal inflammatory responses.

Keywords: Environmental pollutant mixture; low-dose chronic exposure; Inflammatory bowel disease; in vivo model; gut health

Acknowledgments: Funded by Project PRIN 2022 PNRR – ELPME - cod. P2022L82YR_002

A Two-Compartment Microfluidic Device with Integrated Barrier Function Analysis for Investigating Epithelial-Immune Cell Interactions in the Gut

de Hoyos-Vega, Jose M.; Gonzalez-Suarez, Alan M.; Maroli, Gabriel; Cedillo-Alcantar, Diana F.; Dulcini, Alessandro; Le, Hien; Perez, Isaiah; Fedyshyn, Yaroslav; Druliner, Brooke; and Revzin, Alexander

Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

Abstract

Inflammatory Bowel Diseases (IBD), including Ulcerative Colitis (UC), affects nearly 1.3 million individuals in Europe. Interactions between gut epithelial and immune cells are poorly understood, yet these interactions drive IBD pathogenesis and progression. We sought to develop a culture system through which crosstalk between epithelial and immune cell compartments could be tightly controlled. This culture system consists of` two parallel microfluidic chambers that house epithelial and immune cells connected by microgrooves. The epithelial compartment was equipped with a porous membrane and channels bathing apical and basolateral sides. This compartment also integrates chopstick electrodes for making trans epithelial electrical resistance (TEER) measurements. The design of the device facilitates immune cell migration into the epithelial compartment with TEER measurements used for assessing changes in the epithelial barrier function. Intestinal organoids from UC patients were planarized in the microfluidic device. We observed formation of confluent epithelial monolayers in the device over the course of 10 days. Importantly, TEER measurements revealed higher barrier function for normal compared to inflamed intestinal epithelial cells from patient matched organoids. Immunofluorescence staining supported TEER measurements revealing higher levels of tight junction protein ZO-1 expression in normal intestinal epithelial cell layer. As the next step in characterization of the device, we incorporated immune cells in the adjacent compartment and tracked their migration to the epithelial compartment through microgrooves. The system described here will be used to investigate how epithelial-immune cell crosstalk contributes to inflammatory state of the tissue and may also be used for testing molecular or cellular anti-inflammatory therapies.

Keywords: Microfluidics, Organs-on-a-chip, Gut organoids, Trans epithelial electrical resistance (TEER)

Acknowledgments: This project is supported by the Skoll foundation.

Food Toxicity Assessment in the 21st Century: How Far Are We From Alternatives?

Zingales, Veronica; Tolosa, Josefa; Ruiz, María José

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Abstract

One of the central challenges in modern toxicology is to overcome the shortcomings of conventional in vitro studies and animal bioassays and advance toward human-relevant new approach methodologies (NAMs). In line with this goal, the 21st century has seen the emergence of next-generation in vitro testing tools, developed under the 3Rs principles - reduce, replace, and refine – with the aim of better recapitulating the complexity of living systems, improving predictive power, and minimizing reliance on animal models. In this context, considerable progress has already been achieved, with a decisive turning point marked in recent months by an unprecedented synchronicity of policy initiatives that boosted the use of NAMs in biomedical research and preclinical safety assessment. However, important questions remain: how far does this transition reach beyond biomedicine? To what extent does it extend to the toxicological and regulatory evaluation of food contaminants? While several initiatives from regulatory bodies - first of all, the European Food Safety Authority (EFSA) - signal a strong commitment to change, progress in the use of NAMs in food toxicity studies is disappointing. Here, we will provide evidence that European food safety assessment still relies on animal models as the default route for regulatory acceptance. We will further examine the current state of food toxicology, highlighting both emerging efforts and persisting barriers that hinder the implementation of an animal-free testing paradigm. In this context, we will introduce our recent contributions to advancing food safety assessment through 21st century technologies, particularly in the area of mycotoxin risk evaluation. Altogether, these considerations emphasize the urgent need to extend the novel vision of human-relevant toxicology into the field of food safety, since a future with an animal-free science is not only possible, it is imperative. The time for change is now.

Keywords: NAMs; Advanced alternative methods; Food safety, Mycotoxins; Paradigm shift.

Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00 and by the Generalitat Valenciana (Grant CIGE/2024/133). V.Z. acknowledges support from the *Programa Beatriz Galindo* fellowship (BG23/00107) of the Spanish Ministry of Universities.

POSTER COMMUNICATIONS

Characterization of Caco-2 spheroids as a 3D model for the evaluation of Deoxynivalenol (DON) cytotoxicity

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Abstract
Mycotoxins are secondary metabolites produced by filamentous fungi that compromise the safety of food and feed, representing a significant risk to human and animal health. Deoxynivalenol (DON), a type B trichothecene mainly produced by *Fusarium* species, is considered one of the most prevalent mycotoxins globally, commonly contaminating cereals. While cytotoxicity studies of DON and other mycotoxins have traditionally been performed in two-dimensional (2D) *in vitro* cell models, three-dimensional (3D) models based on cellular spheroids have recently emerged as a promising tool for toxicity assessment of food contaminants, as they more accurately reflect *in vivo* cellular architecture and microenvironment. In this work, spheroids of the intestinal cell line Caco-2 were developed under different seeding conditions (with or without collagen) and centrifugation speeds to determine the optimal formation condition. Morphological evaluation was performed for four days of culture by optical microscopy, and parameters such as sphericity index, diameter, and area were quantified using the AnaSP software, specifically designed for spheroid analysis. After four days of growth, spheroids reached an average diameter of 476.2 ± 9.8 µm, an area of 192,441.7 ± 7,940 µm^2, and a sphericity index of 0.9, indicating optimal, compact, and homogeneous structures. Subsequently, cell viability was assessed using the MTT assay after 48h of exposure to DON (2–128µM). The results showed that in the concentration range of 2–64 µM, cell viability remained around 60%, while at the highest concentration tested (120 µM), viability decreased to 40%. Additionally, concentration-dependent morphological alterations were observed, which correlated with the compound's cytotoxic effect. These findings validate the usefulness of Caco-2 spheroids as a robust *in vitro* model for studying mycotoxin-induced intestinal toxicity and provide preliminary data on the effects of DON in a human intestinal 3D system.

Keywords: Deoxynivalenol, 3D models, cytotoxicity, alternative methods

Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00. Martina Sánchez acknowledges the Generalitat Valenciana for the Santiago Grisolia Fellowship (CIGRIS/2023/071).

Bioactive compounds from almond by-products: Functional potential and food safety applications

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Abstract

The growing global consumption of almonds (*Prunus amygdalus*), particularly in Mediterranean regions, generates a variety of by-products that are currently considered waste or used for low-value applications. However, these represent a rich source of bioactive compounds that have great functional potential in the context of the circular economy and agri-food sustainability. This literature review synthesizes current knowledge on the chemical composition, functional properties and potential food safety applications of almond by-products. Multiple studies have identified high levels of bioactive compounds in almond by-products, especially the skin, including (epi)catechin, procyanidins, kaempferol derivatives, isorhamnetin-3-O-rutinoside, catechin and protocatechuic acid. Also, almond hulls and bagasse contain phenolic acids, flavonoids and tannins. These compounds have been associated with antioxidant, anti-inflammatory, antimicrobial, antibiofilm and prebiotic activities. Evidence from diverse experimental models including *in vitro* and *in vivo* assays, food models such as pork patties and *in vitro* digestion-fermentation systems demonstrates effects such as reduced oxidative stress, partial improvement of food shelf life, inhibition of E.coli and S.aureus biofilms and enhanced probiotic functionality after fermentation. In the context of food safety, their potential use as natural mitigators against mycotoxins—a heterogeneous group of toxic secondary metabolites produced by fungi—is being considered, although experimental studies on this topic are still limited. In conclusion, almond by-products, particularly the skin, stand out for their valuable phytochemical profile with great potential for generating functional solutions and innovative applications in the food industry. Their use contributes both to reducing waste and improving food quality and safety, opening up new opportunities for sustainable development.

Keywords: almond, by-products, polyphenols, food safety

Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00. H. Elvira-Pérez is grateful for the collaboration grant (24CO1/004370) awarded by the Ministry of Education. C. Martínez Alonso is grateful for the F.P.U. Scholarship (FPU21/04950) awarded by the Ministry of Education.

Surface-Modified Nanoplastics as Emerging Neurotoxicants: Insights from Human Neuronal Cells

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Abstract

Nanoplastics (NPs) are increasingly present in food, water and air. Once inside the body through ingestion, inhalation or dermal absorption, these particles can reach sensitive organs such as the brain. However, their neurotoxic potential, and particularly the role of surface chemistry in cellular responses, remains poorly understood. In this work, we investigated the impact of three types of 100 nm polystyrene nanoplastics (PS-NPs): unmodified, amine-functionalized and carboxyl-functionalized NPs. Before exposure, the nanoplastic particles were fully characterized and their stability in culture medium was assessed. Human SH-SY5Y neuroblastoma cells were then exposed to concentrations ranging from 1 to 500 µg/mL for 24 or 48 hours. A multi-endpoint approach was applied, combining assays to evaluate cytotoxicity, reactive oxygen species/reactive nitrogen species (ROS/RNS) production, PS-NPs internalization and ultrastructural changes. Functionalized NPs, particularly the amine-modified ones, caused the strongest cytotoxic response, in a concentration- and time-dependent manner, with significant reductions in viability observed at ≥200 µg/mL. Oxidative stress levels increased markedly after exposure to unmodified and amine-modified NPs, especially after 48 hours. Electron microscopy revealed clear signs of cellular damage closely linked to nanoplastic particle surface chemistry, such as dilation of the endoplasmic reticulum, mitochondrial impairment and Golgi fragmentation. Functionalized variants were more efficiently internalized by the neuronal cells, with amine-modified particles showing the highest internalization and signs of lysosomal stress and autophagy. Altogether, these findings highlight surface chemistry as a critical factor shaping the interaction between NPs and neuronal cells. Amine-functionalized NPs stand out for their enhanced internalization, oxidative stress induction and cytotoxicity, underscoring their higher neurotoxic potential and the need to consider surface properties in human health risk assessments.

Keywords: Nanoplastics; Surface functionalization; Neurotoxicity; Human Health risk

Acknowledgments: PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. This work was also supported by FCT/MCTES, through the SALIVA+ project (DOI 705 10.54499/2022.08978.PTDC).

High-Content and Confocal Microscopy Reveal Cytosolic Oxidative Damage Induced by Amitraz in SH-SY5Y 3D spheroid culture

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Abstract

Three-dimensional (3D) spheroid cultures represent a physiologically relevant alternative to conventional two-dimensional (2D) monolayers, providing a microenvironment that better mimics *in vivo* tissue conditions. Their structural complexity enables more reliable assessments of cytotoxicity and oxidative damage. Amitraz (AMZ) is a formamidine acaricide widely used for *Varroa destructor* control in *Apis mellifera*, and its uncontrolled application has led to its detection in honey above permitted levels, posing a potential human health risk. In this work, SH-SY5Y spheroids were used to assess oxidative damage following exposure to AMZ at IC_{50}, IC_{50}/2, and IC_{50}/3 concentrations for 24 h. Cytosolic oxidative stress was detected at IC_{50} conditions through positive CellROX staining, while no mitochondrial involvement was observed (negative PMM and MitoSOX results). Complementary RT-qPCR analysis revealed transcriptional alterations in genes encoding key antioxidant and oxidative stress–related proteins, supporting the selection of catalase and SOD1 for subsequent protein-level evaluation. Indirect immunofluorescence of catalase and SOD1 combined with high-resolution confocal microscopy revealed differential subcellular localization and redistribution patterns of antioxidant enzymes. Quantitative high-content microscopy confirmed a 4-fold increase in catalase spots and a increase in the region/spots ratio compared to vehicle controls (methanol). These findings support the occurrence of cytosolic oxidative damage and an adaptive antioxidant response in SH-SY5Y spheroids exposed to AMZ. The integration of confocal microscopy with high-content imaging provides a complementary and powerful analytical approach. This methodological combination enhances the interpretation of molecular events related to oxidative stress, demonstrating that 3D neuronal models represent robust and sensitive tools for investigating oxidative stress and the toxicological impact of amitraz.

Keywords: Spheroids, Amitraz, High content microscopy, Oxidative stress, Confocal imaging

Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00. F. Franco-Campos acknowledges the “Santiago Grisolía” fellowship (CIGRIS/2021/073).

Towards safer edible insects: assessing contaminant bioaccumulation and depuration capacity in Tenebrio molitor

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Abstract

The integration of insects into sustainable food and feed chains demands a robust evaluation of their capacity to accumulate and eliminate contaminants. Understanding these dynamics is fundamental to ensuring consumer safety and supporting the development of regulatory standards for insect-derived products. This study assessed the bioaccumulation and depuration kinetics of metal(loid)s (As, Cd, Pb) and mycotoxins (Aflatoxin B1, Ochratoxin A, Deoxynivalenol, and Zearalenone) in the yellow mealworm *Tenebrio molitor*, a species already authorized for human consumption in the EU. Larvae were reared on feed substrates artificially spiked at the maximum levels allowed for animal feed under EU legislation. The experimental design included 21 days of exposure followed by a 21-day elimination phase on uncontaminated substrate, to evaluate both uptake and depuration efficiency under realistic production conditions. Distinct toxicokinetic patterns were observed among metals. Arsenic displayed the highest accumulation potential, reaching internal concentrations of approximately 7 mg/kg dry weight (DW) at the end of exposure, with a progressive but incomplete decline during depuration. Cadmium accumulated steadily to ~2.5 mg/kg DW, exceeding the current feed limit, but was rapidly eliminated below regulatory thresholds within one day of clean feeding. Lead, in contrast, showed low assimilation and rapid clearance, remaining consistently below EU maximum levels. Mycotoxins and their metabolites were not detected above quantification limits in any treatment, confirming the species' limited bioaccumulation potential for these compounds. Overall, the results highlight *Tenebrio molitor*'s high depuration capacity and resilience to transient contaminant exposure. These findings highlight the need to apply toxicokinetic principles in insect risk assessment to define effective depuration periods and ensure safe, circular production systems.

Keywords: edible insects; mycotoxins; bioaccumulation; depuration capacity

Acknowledgements: National funds through FCT—Fundação para a Ciência e a Tecnolo gia I.P., under the project/grant UID/50006 + LA/P/0094/2020(doi.org/10.54499/LA/P/0094/2020) and supported by the project ENTOSAFE (PTDC/CTA AMB/0730/2021, https://doi.org/10.54499/PTDC/CTA-AMB/0730/ 2021), financially supported by national funds (OE), through FCT/ MCTES. D. Cardoso is hired under the Scientific Employment Stimulus- Individual Call (CEECIND/01190/2018, https://doi.org/10.54499/CEECIND/01190/2018/CP1559/CT0006).

Analysis of oxidative damage in Caenorhabditis elegans using imaging techniques

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Abstract

The objective of this project was to evaluate, through imaging techniques such as locomotion and fluorescence analysis, the use of *Caenorhabditis elegans* as an indicator of oxidative damage induced by the oxidizing agent hydrogen peroxide (H_2O_2). To achieve it, synchronized adult nematodes were exposed to five different concentrations of H_2O_2 (0, 1, 2, 5, and 7 mM) for 1, 2, and 5 hours. The effect of H_2O_2 was evaluated though locomotion quantification (total movement), the fluorescence of damaged tissues and the lethality analysis (mortality count). The results revealed a concentration- and exposure time- dependent increase in lethality and a decrease in total movement. Fluorescence analysis also demonstrated a clear dose- and time-dependent response, with fluorescence intensity increasing alongside treatment severity. This rise in fluorescence indicated tissue damage caused by oxidative stress from H_2O_2, which was directly associated with nematode lethality and inversely related to movement capacity. In conclusion, the application of locomotion and fluorescence imaging techniques demonstrated that *C. elegans* can serve as an effective biological indicator of oxidative damage induced by the oxidizing agent H_2O_2.

Keywords: Imaging techniques; oxidative stress; hydrogen peroxide; C. elegans; lethality; locomotion; fluorescence

Acknowledgments: The authors gratefully acknowledge the support provided by the “Plan de Recuperación, Transformación y Resiliencia de la Comunitat Valenciana”, through the project “Programa Investigo, Mecanismo de Recuperación y Resiliencia: Subvenciones para la contratación de personas jóvenes demandantes de empleo en la realización de iniciativas de investigación e innovación en la Comunitat Valenciana” (INVEST/2022/24).

Cold plasma for food decontamination: A Meta-review

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Abstract

Cold plasma (CP) is a partially ionized gas generated at room temperature by applying electric fields. The unique composition of plasma leads to the formation of reactive species when applied to food, which have been associated with some beneficial effects. A meta-review of the application of CP in the food sector for the reduction of contaminants was carried out, for which a search was conducted in Pubmed, Scopus, and Web of Science databases of articles published between 2015 and 2025 in English with keywords "Food" AND "Contaminant" AND "Cold" AND "Plasma." A total of 97 articles were found, of which 58 were excluded for not adhering to the scope (reviews focused on reducing contaminants in food). After removing duplicates, a total of 12 articles were included. The studies showed that CP technology is effective in reducing biological and chemical hazards in food. Regarding biological hazards, CP has been shown to be effective in reducing pathogenic bacteria such as *E. coli* or *L. monocytogenes*, among others, in dairy, meat, cereals, and raw vegetables, as well as certain viruses such as *Tulane virus* in fruit and meat samples. For mycotoxigenic fungi, reductions have been observed in both the fungus and spores of the genera *Fusarium*, *Aspergillus* or *Penicillium* in cereals. Concerning chemical contaminants, the application of CP showed the ability to reduce or eliminate aflatoxins, enniatins, and trichothecenes in cereals such as rice, wheat, and barley, as well as in nuts. Other contaminants such as pesticides, especially organophosphates and pyrethroids, were also reduced or eradicated in various matrices, mainly fruits and cereals. Finally, a reduction in certain allergens, such as tropomyosin in shrimp or β-conglycinin and glycinin in soy was also found. In turn, some of the reviews included evaluated the organoleptic and nutritional impact of CP, finding little impact on some matrices such as milk, while in others like spirulina an increase in total phenolic content and carotenoids was observed. This meta-review highlights the usefulness of CP as an emerging technology for reducing both biological and chemical contaminants in food products without compromising their organoleptic or nutritional qualities. However, further study is needed to gain a deeper understanding of its impact on food.

Keywords: Cold plasma, contaminant reduction, emerging technology, mitigation, food safety.

Acknowledgements: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00. A. Navarré is thankful for the ACIF grant (CIACIF/2024/259) provided by the Generalitat Valenciana.

Air-Fryers vs. Deep-Fryers: Acrylamide Formation in French Fries

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Abstract

Air-fryers are increasingly being used as an alternative to conventional deep-fat fryers, as they make it possible to obtain crispy foods with lower fat content and are therefore perceived as a healthier option. However, in the case of French fries, it is important to evaluate how this technology influences the formation of acrylamide, a process contaminant that develops during high-temperature thermal treatments. Moreover, different types of air-fryers are available on the market, varying in the position and design of the heating element that generates and distributes hot air inside the device. These differences could affect heat distribution and, consequently, the amount of acrylamide formed, an aspect that is not yet fully clarified. This study aimed to investigate acrylamide formation in French fries prepared in two different air-fryer models and to compare them with fries prepared in a traditional deep-fryer. Fresh potatoes were cut into strips, fried at 175°C for 5-30 minutes, and then analyzed for color (CIELAB space) and acrylamide concentration (LC-ESI-MS/MS). The results revealed an increase in acrylamide levels and color development that was dependent on frying time across all methods. Deep-fat frying yielded the highest acrylamide concentrations (8922 µg/kg at 30 minutes), whereas air-frying produced substantially lower levels at the same time point (3559 µg/kg and 1316 µg/kg for the two devices tested). Differences in acrylamide formation in French fries prepared with air-fryers may be attributed to variations in design and air-circulation efficiency. These results indicate that air-frying can be an effective strategy to reduce acrylamide levels in French fries made from fresh-cut potatoes. However, since efficiency varies significantly between devices, the heat transmission mechanisms of these appliances should be carefully controlled. These findings may be of commercial interest to air-fryer manufacturers and could support technological improvements aimed at minimizing acrylamide formation.

Keywords: Acrylamide; air-frying; deep-frying; fresh-cut potatoes; food safety

Acknowledgments: This research was funded by Tübitak 2219 International Post Doctoral Research Fellowship Programme for Turkish Citizens.

Study of functional groups with special relevance for the detection of carcinogenicity and mutagenicity of mycotoxins

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Abstract

Toxicological risk assessment aims to identify non-harmful levels of human exposure to specific compounds and chemicals. Special attention must be paid to compounds with carcinogenic potential, as toxicity can occur at very low concentrations. These groups of compounds are included in the known as Cohort of Concern (CoC), which includes three groups of molecules with well-studied and recognized carcinogenic potential: aflatoxins, nitrosamines, and alkyl azoxy compounds. Compounds included in the CoC require special consideration under some regulations, as established by the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH), developing the M7 guideline based on the assessment of impurities that can potentially interact with DNA (mutagenic). In this work we explore two types of computational models for mutagenicity: “rule-based expert systems” (SAR) and statistical models (QSAR), recommend by the ICH-M7 guideline. The main objective was the characterization and generation of structural alerts for the identification of the carcinogenic potential of compounds included in the CoC, concretely aflatoxins, as well as the implementation of a consensus prediction that considers the combination of these alerts with other predictive models of mutagenicity. To this end, specific rules were established to detect compounds that are structurally similar to aflatoxins and that, therefore, should be considered due to their potential carcinogenic and genotoxic potential. To verify the application of these alerts to sets of molecules with a high carcinogenic risk, the presence of SMARTS of 26 functional groups previously identified in the MICOTOXILICO experimental mycotoxin database (https://chemopredictionsuite.com/MicotoXilico; Tolosa et al., 2023) was analyzed. It was observed that, for carcinogenicity, the SMARTS mostly identified in carcinogenic mycotoxins were nNH2, nArylMet and nBycyclic, concluding that these functional groups are particularly relevant for detecting carcinogenicity in natural products.

Keywords: Aflatoxins; Cohort of Concern; Structure-Activity relationship; mutagenicity; carcinogenicity

References: Tolosa, J., Serrano Candelas, E., Valles Pardo, J. L., Goya, A., Moncho, S., Gozalbes, R., & Palomino Schaetzlein, M. (2023). http://doi.10.3390/toxins15060355

Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00 and by the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital, Generalitat Valenciana (CIAEST/2022/35).

Formation of acrylamide and hydroxymethylfurfural in carob flours as affected by roasting temperature: implications for gummy candy production

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Abstract

Gummy candies are popular confectionery products worldwide, although their nutritional value is limited. Reformulating them with functional ingredients could improve their nutritional profile and align with current consumer trends toward healthier foods. Carob flour is a promising ingredient, as it provides fiber, minerals, and bioactive compounds such as D-pinitol, which has antioxidant and antidiabetic properties. However, the roasting process used to obtain carob flour may lead to the formation of potentially toxic compounds, including acrylamide and hydroxymethylfurfural (HMF). This study aimed to evaluate the effect of roasting temperature of carob pods on the acrylamide and HMF content in gummy candies formulated with carob flour. Four carob flours were prepared: unroasted (C) and roasted at 200 °C (T1), 240 °C (T2), and 280 °C (T3) for 195 min. Gummies were elaborated with 5.9 g/100 g carob flour, sucrose, gelatin, and water. Acrylamide and HMF contents were determined by liquid chromatography. Acrylamide was not detected in any of the samples. Conversely, HMF levels increased significantly with roasting temperature: 2.8 mg/100 g (T1), 5.0 mg/100 g (T2), and 22.8 mg/100 g (T3), while no HMF was detected in gummies made with unroasted flour. Although no specific regulatory limits exist for HMF in gummies, comparison with tropical honey (maximum 8 mg/100 g) suggests that samples made with T3 flour may pose a potential risk to consumers. In conclusion, roasting temperatures between 200 and 240 °C represent the upper limit for obtaining carob flour suitable for use in gummy candy formulation, ensuring safety regarding acrylamide and HMF contents while maintaining its functional potential.

Keywords: carob; gummy; roasting temperature; acrylamide; HMF

Acknowledgments: This work was supported by the AGROALNEXT programme (project AGROALNEXT/2022/002), funded by the European Union through the NextGenerationEU initiative (PRTR-C17.I1), the Spanish Government (MCIN), and the Generalitat Valenciana.

Acrylamide levels in biscuits: influence of cereal flour type and baking conditions

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Abstract

In recent years, the demand for whole-grain cereal-based products has increased compared to refined ones, driven by growing consumer awareness of health and nutrition. Additionally, there is rising interest in replacing traditional wheat with alternative cereals, such as rye, to develop formulations with enhanced nutritional profiles and bioactive properties. Although whole grains and alternative cereals offer notable health benefits, their use in high-temperature processing can lead to the formation of harmful compounds like acrylamide, a heat-induced contaminant associated with potential health risks. To address these concerns, this study evaluated acrylamide formation in biscuits prepared from refined wheat flour, whole wheat flour, and whole rye flour, baked under different conditions (160–200°C for 6–14 minutes). Acrylamide levels were strongly influenced by thermal intensity. The highest concentrations were observed in biscuits made with refined wheat flour (355 µg/kg) and whole wheat flour (562 µg/kg) when baked at 200°C for 14 minutes. Conversely, biscuits made with rye flour reached the highest acrylamide content (1454 µg/kg) when baked at 180°C for 14 minutes. None of the biscuits prepared with refined wheat flour exceeded the European benchmark level of 350 µg/kg for acrylamide in biscuits. However, this limit was surpassed in samples made with whole wheat flour, particularly those baked at 180 °C for 14 minutes (512 µg/kg) and at 200 °C for 10 minutes (562 µg/kg). Even higher values were observed in rye flour biscuits baked at lower times and temperatures, reaching 1142 µg/kg after 12 minutes at 180°C and 783 µg/kg after 10 minutes at 190°C. These findings highlight the need to balance nutritional enhancement with food safety when designing cereal-based formulations, especially those incorporating whole-grain flours and alternative cereals. Mitigating the risk of acrylamide formation is crucial to establish optimized baking time–temperature parameters tailored to each product, thereby ensuring consumer safety and regulatory compliance.

Keywords: Acrylamide; Biscuit; Baking conditions; Wholegrain flour; Food safety

Acknowledgments: This research was funded by the project ACRYREVAL (PID2022-137697NB-I00), funded by MCIN/AEI/ 10.13039/501100011033 and by “ERDF A way of making Europe”. The authors gratefully acknowledge financial support from Spanish Ministry of Science, Innovation and Universities through the FPU (Formación de Profesorado Universitario) program.

Mouse Hepatic Organoids as an Alternative Model of Host-Pathogen Interaction

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Abstract

The development of new alternative models is essential to overcome the limitations of traditional twodimensional (2D) cell cultures and animal models. Three-dimensional (3D) models, such as organoids, better mimic the structural and functional complexity of mammal organs, thereby reducing the ethical and economic issues related to animal experimentation. These systems provide more physiologically relevant environments, improving the accuracy of disease modeling and drug response prediction. In this context, we have developed mouse hepatic organoids from livers of adult Balb/c mice, which were co-cultured with Leishmania donovani parasites to establish a model of host-parasite interaction. This model was characterized by RNA-seq linked to differential expression analysis. Interestingly, the presence of the parasites led to a suppression of pathways related to innate immunity, interferon signaling, and iron metabolism, together with cellular stress, damage, or tissue remodelin, with the liver 3D structure attempting to resist or contain the infection through cell proliferation, inhibition of apoptosis, and mechanisms of regeneration or fibrosis. Altogether, these data suggest that the parasite induced an immunosuppressive and pro-parasitic environment in the liver, likely aiding its persistence and replication in the organoid. The findings provided in this work establish mouse liver organoids as an alternative model for pathogenesis studies.

Keywords: hepatic organoids, pathogenesis, co-culture, Leishmania.

Influence of post-harvest methods and roasting intensity on thermal processing contaminants in coffee

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Abstract

Coffee is one of the most consumed beverages worldwide, and its quality is largely influenced by post-harvest processing and roasting. Post-harvest treatments aim to remove the pulp from the cherries to obtain “parchment coffee,” with dry, semi-dry, and wet processing being the most common post-harvest methods. Once parchment coffee is obtained, a hulling step is carried out to remove the parchment from the bean, and then the beans are processed for roasting. Roasting is essential for developing the desirable aroma and flavor of coffee; however, it also promotes the formation of undesirable thermal contaminants through Maillard and sugar dehydration reactions, such as acrylamide and furanic compounds, particularly 5-hydroxymethylfurfural (5-HMF). Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2A) and associated with adverse effects on the nervous and reproductive systems. Studies have shown that 5-HMF acts as a critical intermediate in acrylamide formation, so controlling 5-HMF levels could serve as an effective strategy to mitigate acrylamide formation. This work aimed to evaluate the effect of post-harvest treatments (dry, semi-dry, and wet) and roasting degree (green (no roasting), medium roast, and dark roast) on 5-HMF formation in *Coffea arabica* L. var. Colombia. The 5-HMF content was quantified on ground coffee samples from each treatment and roasting level by isocratic reversed-phase HPLC analysis. Results showed that both post-harvest method and roasting degree significantly influenced 5-HMF content. The semi-dry process produced the highest 5-HMF concentrations, while dark roasting yielded markedly higher values than medium roasting. These findings suggest that optimizing post-harvest processing and controlling roasting conditions could therefore be an effective strategy to reduce 5-HMF levels and, consequently, mitigate acrylamide formation, contributing to safer and higher-quality coffee products.

Keywords: Coffee; Post-harvest; Roasting; Acrylamide; 5-hydroxymethylfurfural

Bioactive compounds and mineral bioaccessibility of solid and liquid fractions obtained after the lactic acid bacteria (L. plantarum and L. casei) fermentation of SC-CO2-defatted spirulina through an in vitro simulated gastrointestinal digestion

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Abstract

Spirulina (*Arthrospira platensis*), a cyanobacterium widely recognized for its high protein content and dense nutritional profile, represented a particularly promising substrate for bioprocessing. The supercritical carbon dioxide defatted spirulina (SC-D-*Sp*) was obtained through the SC-CO_2 process (25.0 MPa, 50.0 ℃, 16.0 mL/min with CO_2/EtOH=90:10, and extraction time of 60.0 min) to enhance the relative concentration of proteins and minerals, making it an attractive material for spirulina valorization. Consequently, the SC-D-*Sp* was used as a substrate for 5% lactic acid bacteria (*Lactobacillus plantarum*, LP, and *Lactobacillus casei*, LC) fermentation. While extraction and fermentation provide valuable compounds, their nutritional significance ultimately depends on gastrointestinal bioaccessibility. Then, the fermented fractions (solids and supernatants) were subjected to *in vitro* digestion, and the resulting digesta were analyzed for protein, bioactive peptides (BPs), minerals, and antioxidant capacity (TAC). Protein concentrations in the digested supernatants (control, without fermentation, 5%LP, and 5%LC) were significantly higher than those in the digested solid fractions. Bioactive peptides exhibited angiotensin-converting enzyme (ACE) inhibitory, antioxidant, antibacterial, hypotensive, antithrombotic, and binding activities. Mineral bioaccessibility (Mg, P, K, Fe, Cu, and Se) was also significantly higher in the digest-supernatant. The digest-supernatant-5%LP and digest-supernatant-5%LC demonstrated notably high TAC values. Principal component analysis (PCA) revealed that the digest-supernatant (5% LP and 5% LC) confirmed their superior release of BPs, antioxidants, and bioaccessible minerals.

Keyworks：SC-CO_2 defatted spirulina (SC-D-Sp); in vitro; lactic acid bacteria (LAB); bioactive peptides (BPs); bioaccessibility.

Reference: Liu, Y., Castagnini, J. M., Berrada, H., & Barba, F. J. (2025). https://doi.org/10.1016/j.foodchem.2025.143085

Acknowledgments: The publication is part of the BLUEWAYSE "BLUE WAY to a Sustainable Europe" action PCI2024-153460 (Sustainable Blue Economy Partnership, SBEP), funded by MICIU/AEI/10.13039/501100011033 and by the European Union under grant agreement nº: SBEP2023-961. Yixuan Liu is supported by a Ph.D. fellowship from the China Scholarship Council (CSC) (No. 202208420013).

Antifungal and Anti-Mycotoxigenic Effects of Carob (Ceratonia siliqua L.) Extracts against Zearalenone-Produced by Fusarium Species

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Abstract

Zearalenone (ZEN), a polyketide-derived mycotoxin produced by *Fusarium* species, is a major contaminant in cereals and derived food products. It exerts estrogenic and cytotoxic effects mainly through oxidative stress induction and endocrine disruption, triggering apoptosis and genotoxicity. Its metabolites, α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), have also demonstrated neurotoxic potential in human and animal cellular models (Tatay *et al.,* 2018). Among mitigation strategies, plant-based and biological treatments are increasingly considered safe and sustainable alternatives for mycotoxin control (Murtaza *et al.*, 2023). The carob tree (*Ceratonia siliqua* L.), widely distributed in the Mediterranean, is rich in phenolic acids, flavonoids, and tannins, which are responsible for its antioxidant, antimicrobial, and antiproliferative properties (Kavvoura *et al.*, 2023). Ethanolic and aqueous extracts of carob pods exhibit notable antifungal activity against toxicogenic species such as *Fusarium graminearum* and *Aspergillus flavus*, acting mainly through membrane disruption and sporulation inhibition. The antifungal potential is enhanced at higher extract concentrations due to greater solubilization of bioactive phenolic compounds (Baba Ahmed *et al.*, 2023). The anti-mycotoxigenic potential of carob and other plant extracts has been confirmed by several recent studies. Hou *et al.* (2023) reported that polyphenolic compounds inhibited *Fusarium graminearum* growth and ZEN biosynthesis through downregulation of PKS genes, while Murtaza *et al.* (2023) found that rhizobacterial phenolics reduced ZEN levels by modulating oxidative-stress pathways. Similarly, extracts with higher antioxidant capacity, quantified by HPLC–LC–MS/MS, achieved greater suppression of ZEN accumulation. Together, these findings support the role of phenolic antioxidants as dual antifungal and anti-mycotoxigenic agents.

Keywords: Ceratonia siliqua L.; Zearalenone (ZEN); Antifungal activity; Phenolic compounds.

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Acknowledgments: This research was funded by Tunisian Ministry of Higher Education and Scientific Research and MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00.

Evaluation of the Preventive Effect of Cork Oak (Quercus suber L.) Acorn Flour against Aflatoxin B1 Toxicity In Vivo

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Abstract

Aflatoxin B1 (AFB1) produced by *Aspergillus flavus* and *A. parasiticus* is a highly potent mycotoxin classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen. It poses serious public health and food safety risks due to its hepatotoxic, nephrotoxic, and immunosuppressive effects, primarily mediated through oxidative stress and organ dysfunction (Tolosa *et al.,* 2021). This study investigated the protective role of cork oak acorn flour (COAF) at two dietary doses (5% and 10% integrated into the industrial granule) against AFB1-induced toxicity in male Balb/c mice for two weeks. Eight groups were included: control, received (5% or 10% of COAF only), pre-treatment with 5% or 10% of COAF then AFB1, co-treatment 5% or 10% of COAF with AFB1, and AFB1 group (100 µg/kg AFB1 in PBS by oral route). Body weight, food intake, and several biochemical markers were analyzed. Total protein was measured using the Bradford method, sulfhydryl proteins were quantified following the Sedlak and Lindsay method, catalase activity was assessed according to Aebi, and malondialdehyde (MDA) levels were determined using the Yagi method, along with histological evaluation of liver and kidney tissues. AFB1 exposure significantly increased MDA levels while reducing protein thiols and catalase activity, indicating oxidative stress and metabolic imbalance. Supplementation with COAF, particularly at 10%, effectively mitigated these effects by lowering MDA levels, restoring antioxidant enzyme activity, and improving biochemical and histological parameters. **The** pre- and co-treated groups exhibited nearly normal hepatic and renal structures compared to the severe degenerative lesions in the AFB1 group, consistent with Ali *et al.* (2021). Overall, oak acorn flour, especially at 10%, exhibited strong protective activity against AFB1-induced oxidative damage, supporting its potential as a natural antioxidant source (Cheng *et al.,* 2023). Future work should isolate and characterize the active antioxidant compounds in oak acorns for potential use in functional foods.

Keywords: Quercus suber.l; Aflatoxin B1 (AFB1); Toxicity; Oxidative stress; Food safety.

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Acknowledgments: This research was funded by the Tunisian ministry of Higher education and scientific and MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00.

Shared molecular mechanisms between Alzheimer's disease and mycotoxin-induced neurotoxicity

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Abstract

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive synaptic dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, and neuronal apoptosis[1]. Beyond its well-known genetic and age-related risk factors, increasing evidence supports a significant contribution of environmental toxicants to AD pathogenesis. Among these, mycotoxins, fungal secondary metabolites frequently detected in food and feed, have emerged as potential environmental cofactors with neurotoxic potential[2]. This review identifies and compares the molecular mechanisms shared by AD and mycotoxin-induced neurotoxicity. A systematic literature search was performed in PubMed, Scopus, Google scholar and Web of Science databases covering the period 2018–2025, using the keywords *mycotoxin*, *neurotoxicity*, *Alzheimer's disease*, *oxidative stress*, *inflammation*, *apoptosis*, *tau*, *amyloid-beta and microglial activation*. Eligible studies included original research and reviews assessing neuronal or brain-related models exposed to major neurotoxic mycotoxins (ochratoxin A (OTA), fumonisin B1 (FB1), and T-2 toxin. Data were extracted regarding model type, exposure conditions, molecular pathways affected, and biomarkers analyzed. Across the selected literature, OTA and FB1 were the most extensively studied in neuronal models such as SH-SY5Y, PC12, immortalized-human astrocytes (IM-HA) and primary cortical neurons. Both toxins consistently induced reactive oxygen species (ROS) generation, mitochondrial dysfunction, and apoptosis**,** mediated by p53 activation, Bax/Bcl-2 imbalance, and caspase-3 cleavage[3,4]. Several reports also demonstrated NF-κB–dependent inflammatory signaling and downregulation of the Nrf2/HO-1 antioxidant pathway[5]. These processes mirror central mechanisms of AD, where oxidative stress and chronic inflammation promote GSK3β activation, tau hyperphosphorylation, and amyloid-beta aggregation[6]. Although direct evidence linking chronic mycotoxin exposure to Aβ or tau pathology remains limited, the convergence of oxidative, inflammatory, and apoptotic cascades suggests a shared molecular landscape. Overall, these findings highlight that mycotoxin-induced neurotoxicity reproduces several hallmarks of AD, reinforcing the hypothesis that mycotoxins may act as environmental contributors to neurodegenerative disorders.

Keywords: Alzheimer's disease, mycotoxins, oxidative stress.

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Acknowledgments: This research was funded by MICIU /AEI /10.13039/501100011033 / FEDER, UE through the project PID2024-160527OB-I00.

Organizer:
University of Valencia research group (GIUV2021-513)

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