ESTRATEGIAS BIOTECNOLÓGICAS PARA REDUCIR EL CRECIMIENTO DE HONGOS TOXIGÉNICOS EN ALIMENTOS

PROYECTO PROMETEO 2018-2022

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Estrategias biotecnológicas para reducir el crecimiento de hongos toxigénicos en alimentos.

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Sobre el proyecto

La bioconservación es una aplicación biotecnológica que consiste en el empleo de microorganismos o sus productos metabólicos en alimentos para inhibir el crecimiento microbiano, con el objetivo de mejorar la seguridad alimentaria y extender la vida útil de los productos alimenticios. El deterioro de los alimentos causado por hongos toxigénicos es uno de los retos más importantes actualmente en seguridad alimentaria debido al incremento del comercio internacional de alimentos. En este contexto, las cepas de bacterias ácido lácticas (BAL) y la harina de mostaza amarilla son capaces de aportar compuestos antifúngicos. El empleo de ingredientes naturales, obtenidos a partir de productos del metabolismo de las BALs y de las crucíferas pueden ser una buena herramienta para promover la seguridad alimentaria y reducir el uso masivo de aditivos de síntesis.

El objetivo del presente proyecto es estudiar las propiedades antifúngicas y antitoxigénicas de los metabolitos producidos por bacterias lácticas y de la harina de mostaza amarilla como elementos susceptibles de ser utilizados para la conservación del pan de molde y del queso fresco y, en consecuencia, aumentar la vida útil de los mismos. Todo ello si la evaluación del riesgo así lo aconseja, para lo que se realizarán ensayos de bioaccesibilidad, biodisponibilidad y toxicidad *in vitro* tanto de los compuestos antimicrobianos producidos por las BALs como por las moléculas antimicrobianas que caracterizan la actividad de la harina de mostaza amarilla.

Dr. Jordi Mañes Vinuesa

Programa científico

9:30- 9.45 – INAUGURACIÓN DE LAS JORNADAS	
Sesión I	**10/12/20**
9.45 -10:00	**EVALUATION OF BUFALA WHEY MILK FERMENTED BY LACTIC ACID BACTERIA AS A BREAD BIOPRESERVATIVE AGENT** *C. Luz, V. Dopazo, J. Calpe, J.M. Quiles, J. Mañes, G. Meca* Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain
10.00 -10:15	**BIOACCESSIBILITY AND BIOAVAILABILITY OF PHENOLIC ACIDS FROM FOOD EXTRACTS FERMENTED WITH LACTIC ACID BACTERIA** *L. Escrivá, A. Marucci, C. Luz, J. Mañes, G. Meca, L. Manyes* Departamento de Medicina Preventiva y Salud Pública, Ciencias de la Alimentación, Toxicología, y Medicina Legal. Facultad de Farmacia. Universitat de València.
10:15-10:30	**BIOPRESRVATION POTENTIAL OF LACTIC ACID BACTERIA AGAINST PATHOGENIC FUNGI ON RED GRAPE** *V. Dopazo, C. Luz, J.M. Quiles, R. Carbonell, J. Mañes, G. Meca* Laboratory of Food Chemistry and Toxicology. Faculty of Pharmacy. University of Valencia. Spain
10:30-10:45	**TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN FERMENTED FOOD EXTRACTS AFTER SIMULATED DIGESTION AND *IN VITRO* ABSORPTION IN CACO-2 CELLS** *A. Marucci, L. Manyes, G. Meca, G. Font, L. Escrivá* Departamento de Medicina Preventiva y Salud Pública, Ciencias de la Alimentación, Toxicología, y Medicina Legal. Facultad de Farmacia. Universitat de València.
10:45-11:00	**EXPERIMENTAL DESIGN TO ASSESS PROTECTIVE ROLE OF MILK FERMENTED WHEY AND CAROTENOIDS AGAINST AFB1 AND OTA INDUCED TOXICITY IN JURKAT CELLS** *M. Corbella, A. Cimbalo, L. Escrivá, G. Font, L. Manyes* Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Burjassot, Spain.
11:00-11:15	**ASSESSMENT OF THE BIOACCESSIBILITY OF AFLATOXIN B_1 AND OCHRATOXIN A FROM WHEAT BREAD BY IN VITRO DIGESTION** *R. Pietrzak-Fiecko[1], P. Llorens[*2], L. Pollini[3], J. Mañes[2], C. Juan[2]* [1]Department of Commodities and Food Analysis, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn (Poland); [2]Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia (Spain); [3]Food Science and Nutrition Section Department of Pharmaceutical Sciences, University of Perugia (Italy)
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09:30-09:45	**ANTIFUNGAL ACTIVITY OF PERACETIC ACID AGAINST *ASPERGILLUS FLAVUS* IN CORN** ***J.M. Quiles, R. Carbonell, J. Manyes, G. Meca*** **Av. Vicent Andrés Estellés s/n, 46100 Burjassot (Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia) Spain**
09:45-10:00	**SWITCHING ENERGY METABOLISM INCREASES SUSCEPTIBILITY OF SH-SY5Y CELLS TO *STERIGMATOCYSTIN*: A MITOCHONDRIAL TOXIN** *V. Zingales, M. Fernández-Franzón, MJ. Ruiz* Universitat de València. Facultat de Farmàcia. Laboratorio de Toxicología. Av. Vicente Andrés Estellés s/n 46100 Burjassot, Valencia, España.
10:00-10:15	**DOES MYCOTOXIN EXPOSURE ALTER BRAIN CELLS DIFFERENTIATION IN VITRO? PROTOCOL DESIGN AND OPTIMIZATION USING SH-SY5Y CELL LINE.** *M. Frangiamone, M.Alonso-Garrido, G. Font, L. Manyes.* Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, Universitat de València, Burjassot, Spain.
10:15-10:30	**STUDY OF ENZYMATIC DEFENSE SYSTEM IN NEUROBLASTOMA CELLS EXPOSED TO ZEARALENONE'S METABOLITES AND BEAUVERICIN** *F. Agahi, A. Juan-García, C. Juan* Laboratory of Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vincent Andres Estelles s/n 46100 Burjassot, Valencia, Spain
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	Importaco Frutos Secos: "*Micotoxin risk management in food industry". Dra.* Amparo Devesa. Directora Científica de Calidad, Medioambiente e Investigación.
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Presentaciones orales

EVALUATION OF BUFALA WHEY MILK FERMENTED BY LACTIC ACID BACTERIA AS A BREAD BIOPRESERVATIVE AGENT

C. Luz, V. Dopazo, J. Calpe, J.M. Quiles, J. Mañes, G. Meca

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The aim of this study was to revaluate Mozzarella di Bufala Campana whey fermentation by lactic acid bacteria (LAB) and the use of this ingredient as a bio-preservative in bread production. Whey was pasteurized and fermented by nine selected LAB with antifungal activity for 72 h at 37 ºC. Subsequently, fermented whey (BWF) was incorporated into the bread formulation, and the pH; antimicrobial metabolites, such as organic acids and volatile organic compounds (VOCs); total phenolic content; DPPH radical-scavenging activity and visual shelf-life were characterised. The highest lactic acid content was observed in the BWF by *L. plantarum* TR7 (15.0 g/L) and *L. plantarum* TR2 (12.5 g/L). In addition, an increase in VOCs such as a hexanal, benzeneacetaldehyde, benzaldehyde and pyrazine tetramethyl was determined in bread with BWF. BWF by LAB evidenced an increase in radical scavengers, and this was reflected in a 33% rise in the DPPH- inhibitory activity of bread with BWF compared with the control. Breads in which 100% of the water was replaced with BWF by *L. plantarum* TR7 and *L. ghanensis* TR2 showed fungal growth at 20 days of storage and evidenced an improvement in the shelf-life by 2 and 15 days compared with a control containing calcium propionate at 0.3% and control bread, respectively.

Keywords: Mozzarella di Bufala Campana whey, lactic acid bacteria, biopreservation, bread shelf-life.

BIOACCESSIBILITY AND BIOAVAILABILITY OF PHENOLIC ACIDS FROM FOOD EXTRACTS FERMENTED WITH LACTIC ACID BACTERIA

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Microbial fermentations with lactic acid bacteria (LAB) produce bioactive compounds, including phenolic acids, that released during human digestion, may have antioxidant, antimicrobial and antifungal activity against several mycotoxigenic fungi. The objective of the present study was to evaluate the bioaccessibility and bioavailability of phenolic acids from two food matrices -whey powder and yellow mustard flour- fermented with *Lactobacillus plantarum*, respectively. Fermented and non-fermented extracts were analysed by LC-qTOF-MS (*initial extracts*). For bioaccessibility determination each extract was subjected to a simulated human gastrodigestion system reproducing physiological steps (oral, gastric and pancreatic digestion) by incubation at 37.5°C with i) artificial saliva containing α-amylase; pepsin (pH=2; 2h incubation); and ii) pancreatin and bile salts (pH=6.5; 2h incubation). Intestinal digest (pH=7.2) was analysed by LC-qTOF-MS (*digested extracts*), and frozen (-80°C), lyophilized, and resuspended in HBSS. Bioavailability evaluation was performed on *in vitro* intestinal epithelium Caco-2 cells model cultured in Transwell plates (225000 cells/well) until complete differentiation (day 21; verified by the neutral red assay). Four concentrations of digested extracts (0.2, 0.4, 0.6 and 0.8%) were added in triplicates into the apical area, and aliquots of both apical and basolateral compartments were collected at different times (0, 1, 2, 3, 4h) and analysed by LC-qTOF-MS (*absorbed extracts*). Analysis of initial extracts showed the presence of phenolic acids including DL-3-Phenyllactic acid and Lactic acid in whey and mustard extracts; while Benzoic acid, P-Coumaric acid, and Sinapic acid were only present in mustard extracts. Preliminary results showed bioaccesibility of DL-3-Phenyllactic ranging between 2.8-6.0% and 1.2-3%, in mustard and whey extracts, respectively; being slightly higher in the case of fermented whey compared to the non-fermented extract. The analysis of collected apical and basolateral aliquots will allow the study of the human absorption process, as well as the bioavailability determination of these phenolic compounds.

Keywords: phenolic acids, lactic acid bacteria, bioaccesibility, bioavailability, Caco-2 cells

BIOPRESERVATION POTENTIAL OF LACTIC ACID BACTERIA AGAINST PATHOGENIC FUNGI ON RED GRAPE

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Nowadays, the increasing resistance of fungi to pesticides is a concerning issue to the industry. New antifungal methods are being proved, such as the use of microorganism for biopreservation. In this study, the biopreservation potential of four strains of *L. plantarum* was tested against fungi from *Aspergillus* and *Botrytis*. To achieve this objective, a characterization of the antifungal activity of the bacterial fermented Man–Rogosa–Sharpe (MRS) medium was performed. Also, the analysis of different compounds presents in the fermented medium. In the end, all four fermented mediums were tested as biopreservation of red grapes contaminated with *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus tubingensis* and *Botrytis cinerea*. The antifungal activity results showed that *L. plantarum* E3 and *L. plantarum* E4 exhibited the highest antifungal activities reaching minimum fungicidal concentrations from 6.3 to 100 g/L. The analysis of the compounds evidenced a wide pool of different antifungal molecules in the CFS's that were not present in the non-fermented medium. Such as acid lactic, acetic acid, phenyllactic acid, dihydrocaffeic acid, benzoic acid ketones and pyrazines. Finally, in the test performed on red grapes all four CFS evidenced a significant reduction of 1.32 Log_{10} spores per gram of fruit in the grapes contaminated by *A. ochraceous* compared to the MRS medium control. Likewise, CFS produced by *L. plantarum* E3 evidenced a reduction of 0.92 Log_{10} spores per gram of fruit for the grapes contaminated by *B. cinerea*.

Keywords: Lactic acid bacteria, *Botrytis*, *Aspergillus*, Biopreservation.

TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN FERMENTED FOOD EXTRACTS AFTER SIMULATED DIGESTION AND IN VITRO ABSORPTION IN CACO-2 CELLS

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Fermentation with lactic acid bacteria (LAB) is studied as strategy to decrease fungal contamination and mycotoxins production since originates bioactive compounds, such as polyphenols, with antimicrobial, antifungal and antioxidant activity. The aim of the present study was to evaluate total polyphenols and antioxidant activity of mustard flour and whey extracts fermented with LAB after simulated human digestion and *in vitro* absorption in Caco-2 cells. Fermented and non-fermented extracts were subjected to a digestion process in different steps by enzymes addition (α-amylase, pepsin; pancreatin, bile salts), pH adjustment (pH=2; 6.5; 7.2) and incubation (37.5ºC; 4h). Digested extracts were lyophilized and resuspended at different concentrations (0.2, 0.4, 0.6 and 0.8%) in HBSS. Extracts were added into the apical part of the intestinal epithelium model once the intestinal barrier was completely formed (differentiation day 21). Cells were incubated 4h and both apical and basolateral aliquots were collected at 1, 2, 3, 4h to be analysed. Total polyphenols were determined by Folin-Ciocalteu Reagent microprocedure. Briefly, 25 µL sample (or gallic acid as standard curve) were mixed with 125 µL diluted Folin (1/5), and 25µL NaCO3 (20%) after vigorous agitation. Absorbance was measured at 750 nm after 1h incubation in darkness. Antioxidant activity was determined by the reduction of 100 µL 2,2-diphenyl-1-picrylhydrazyl (DPPH) after its reaction with 50 µL sample, 1h incubation in darkness and absorbance measurement at 517 nm. Preliminary results showed increased concentration of total polyphenols with dose increasement in flour extracts collected at 2, 3 and 4h, with values ranging between 0.3 and 20.8 mg/L. Whey extracts showed detectable polyphenols concentrations only at the highest doses (0.6, 0.8%) and at 3, 4h (0.5-1.2 mg/L). Low antioxidant activity was observed (<6%) mainly at 3, 4h. Results indicate that polyphenols and antioxidant compounds are present in small amounts in mustard flour and whey extracts after simulated human digestion and absorption.

Keywords: antioxidant activity, total polyphenols, lactic acid bacteria, intestinal absorption

EXPERIMENTAL DESIGN TO ASSESS PROTECTIVE ROLE OF MILK FERMENTED WHEY AND CAROTENOIDS AGAINST AFB1 AND OTA INDUCED TOXICITY IN JURKAT CELLS

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Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) are mycotoxins produced by filamentous fungi belonging to *Aspergillus* and *Penicillium* genera. They are currently considered the most important mycotoxins in terms of food safety, in both humans and animals. During the years, it has been investigated the ability of bioactive compounds to prevent mycotoxins adverse effects. For this purpose, the beneficial effect of milk fermented whey and pumpkin extract rich in carotenoids on AFB1 and OTA cytotoxicity will be evaluated in Jurkat T cells through a proteomic approach. Jurkat T cell culture exposed to: a) AFB1, b) OTA, c) AFB1 and OTA, d) fermented whey, e) carotenoids, f) fermented whey and carotenoids, g) AFB1, OTA and fermented whey h) AFB1, OTA and carotenoids i) AFB1, OTA, fermented whey and carotenoids and to control DMSO 0.5%. Proteins will be extracted from exposed cells by means of a lysis buffer (Urea 8M / Thiourea 2M / Tris-HCl 50mM) and subsequently quantified by using a NeoDot nano-spectrophotometer. Afterwards, a concentration of 1000 ppm protein extract will be reduced with dithiothreitol and alkylated with iodoacetamide at a concentration of 200 mM in order to disrupt polypeptide chains. Lastly, peptides will split in Lysine-Arginine bonds through a tryptic digestion overnight. Three biological and two technical replicates of each sample will be analyzed with an LC system coupled with quadrupole time of flight (Q-TOF, Agilent) in a concentration of 100 μg/μL by using a C18 column during a 40 minutes run time at a flow rate of 0.5 mL/min. The data obtained will be processed with Sprectrum Mill software and the differentially expressed proteins will be statistically evaluated by using Mass Professional Profiler software (Agilent). Results will show significant differentially expressed proteins involved in Jurkat T cell functions and the impact which they may produce on human health.

Acknowledgments: *This work was supported by Spanish Ministry of Economy and Competitiveness (PID2019-108070RB-I00-ALI) and grant (GVPROMETEO2018-126).*

Keywords: mycotoxin, proteomics, lymphoblastoid cells, Q-TOF, prevention

ASSESSMENT OF THE BIOACCESSIBILITY OF AFLATOXIN B_1 AND OCHRATOXIN A FROM WHEAT BREAD BY *IN VITRO* DIGESTION

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Bread has been one of the world's most important foods and it is susceptible to the action of moulds. Access of the mycotoxigenic fungi to the raw materials and the finished product, should therefore be restricted through proper storage, conditioning of the flour, and an adequate indoor air quality system. Mycotoxins are the most important contaminants in cereals and related products, in terms of economic effect and toxicity. In the evaluation of the oral bioavailability of mycotoxins, the first step is the determination of its bioaccessibility.

The present study aims to investigate the bioaccessibility of AFB_1 and OTA from wheat bread using an *in vitro* digestion model under fed conditions. The digestion model consists of initial saliva processing for 5 min at 37°C to simulate the mouth compartment and the gastric conditions for 2h, followed by simulated small intestine compartment for 2h at 37°C (Minekus et al., 2014). Afs&OTA-free samples were spiked with AFB_1 and OTA at two levels (10 and 5 μg/g) in single and combination. The extraction procedure was based on a mixture of acetonitrile/water (84/16, v/v) as described by Juan et al. (2013). Analytes were determinate by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS). The bioaccessibility from bread matrices ranged from 68 to 45% for OTA, and 40 to 34% for AFB_1. AFB_1´s results were similar and slightly higher than observed in previous studies 54 and 26% (Saladino et al., 2018). It highlights that there are few studies on AFB_1 and OTA bioaccessibility, which are valuable to correlate with the real exposure risks and permits to conclude bioavalability studies. Thereforce, the present study is included in a current in progress bioavalability study.

1 Juan C., et al. (2013). Food Chemistry. 141(3):1747–1755
2 Minekus M., et al. (2014). Food and Function. 5(6): 1113–1124.
3 Saladino F., et al. (2018). Journal of Food Composition and Analysis. 68, 128–132.

Keywords: bread, mycotoxins, digestion, OTA, AFB_1

ANTIFUNGAL ACTIVITY OF PERACETIC ACID AGAINST ASPERGILLUS FLAVUS IN CORN

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Peracetic acid (APA) is a compound used in the food industry for the disinfection of food and food-contact surfaces. Its mechanism of action is to oxidize lipid membranes, DNA, metabolites and proteins with sulfhydryl groups and double bonds. Its main advantages are its wide antimicrobial spectrum and that its decomposition products (CH_3COOH, O_2 and H_2O) are substances with low toxicity.

This study aims to quantify the antifungal activity of APA against the toxigenic fungi *Aspergillus Flavus* (*A. flavus*), a common corn contaminant. First, the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of APA were established against A. flavus in liquid medium, using the 96-well plate method. Secondly, the use of APA released through a hydroxyethyl cellulose (HEC) gel in 1L jars was tested to inhibit the growth of different concentrations of *A. flavus* inoculated into PDA solid media plates. Micellar growth was observed for 10 days. Finally, the same methodology was used to evaluate the antifungal activity and the reduction of aflatoxin B1 (AFB1) production caused by the APA-releasing HEC gel in *A. flavus* contaminated corn. Fungal growth was studied by colony counting in PDA plates seeded with decimal serial dilutions of contaminated corn; AFB1 production was determined in methanol extracted samples of contaminated corn analyzed by mass spectrometry-associated liquid chromatography (HPLC-MS/MS).

The results of MIC and MFC for APA against *A. flavus* in liquid medium were of 125 mg/L and 187.5 mg/L, respectively. The antifungal doses for APA released through an HEC gel were estimated in a range between 10 mg/L and 25 mg/L for *A. flavus* inoculated in PDA plates and 300 mg/L for the contaminated corn, where this dose was also able to completely reduce AFB1 production.

Keywords: Aflatoxin B1, antifungal activity, *Aspergillus flavus*, Minimal Inhibitory Concentration, Minimal Fungicide Concentration.

SWITCHING ENERGY METABOLISM INCREASES SUSCEPTIBILITY OF SH-SY5Y CELLS TO STERIGMATOCYSTIN: A MITOCHONDRIAL TOXIN

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Mitochondria are key cellular organelles known to guarantee many physiological processes, such as energy production through the oxidative phosphorylation process (OXPHOS). In *in vitro* conditions, cell lines are metabolically adapted to growth rapidly and, for this reason, they derive most of their energy from glycolysis rather than OXPHOS, a phenomenon known as the Crabtree effect. The substitution of galactose for glucose in the culture medium is an expeditious way to reverse the Crabtree effect and determine mitochondrial toxicity. The aim of the present study was to evaluate the role of mitochondria in the toxicity induced by the mycotoxin sterigmatocystin (STE) on human neuroblastoma SH-SY5Y cells. Cells were cultured in presence of glucose (25 mM) or galactose (10 mM) as the only sugar available. The effects on cell viability were evaluated by MTT assay. The change in the fuel source caused a cell viability decrease on galactose-grown cells compared to glucose-grown cells, suggesting that STE exhibited an increased level of toxicity in SH-SY5Y cells following the switch to OXPHOS. Furthermore, considering the crucial importance of a functional electron transport chain (ETC) in OXPHOS conditions, we also compared the effect of STE exposure in the presence or not of known ETC inhibitors (antimycin A and rotenone) in cells grown in galactose-supplemented medium. Treatment with STE and rotenone, a selective inhibitor of the complex I, resulted in a further significant decrease in cell viability respect to cells only exposed to the mycotoxin, while no effect was observed in the presence of antimycin A, an inhibitor of the complex III. These data highlight that STE might affect the complex I, whereas the complex III of the ETC seems to be not involved in STE toxicity. Taken together, our results suggest that the etiology of STE cytotoxicity may depend on mitochondrial impairment.

Acknowledgments: *This research has been supported by the Generalitat Valenciana grant (Prometeo 2018/216) and the pre-doctoral research training program "Santiago Grisolia (GRISOLIAP/2018/092) CPI-18-117".*

Keywords: Sterigmatocystin, SH-SY5Y cells, Cytotoxicity, Mitochondria, Crabtree effect.

DOES MYCOTOXIN EXPOSURE ALTER BRAIN CELLS DIFFERENTIATION *IN VITRO*? PROTOCOL DESIGN AND OPTIMIZATION USING SH-SY5Y CELL LINE.

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Human SH-SY5Y neuroblastoma cells represent a suitable *in vitro* model to investigate toxicity in the brain and reproduce accurately neurodegenerative diseases. Although SH-SY5Y cells are widely used in neuronal research, they are epithelial cells with no neuronal proprieties unless they are treated with retinoic acid (RA) and differentiated into dopaminergic neurons. Thus, neuronal features, discriminating undifferentiated and RA-differentiated SH-SY5Y cells and showing significant differences between these cell models, will be characterized. In this regard, two different techniques, flow cytometry and microscopy, are being implemented to highlight morphological and functional changes induced by differentiation. Cells will be exposed to several mycotoxins concentrations, individually and in combination, and the exposure will start at different differentiation time points in order to assess the risk of exposure to theses food contaminants. Flow cytometry experiment allows to outline differences in cell cycle, since differentiated cells featured a significant decrease in the proliferation rates, mainly associated with a decrease in S phase in combination with an arrest in G2-M. In this case, DNA composition will be analyzed using propidium iodide. Instead, optical microscopy enables to analyze the cell morphology since RA-differentiated cells showed an increased neurite density, suggesting a change from epithelial to a stellate neuronal morphology. Moreover, immunofluorescence microscopy permits to detect the dopamine content, potentiated by RA-differentiation. Neurites density and dopamine reactivity will be evaluated using an anti-bIII tubulin and anti-dopamine antibodies respectively, followed by incubation with their Alexa 488- conjugated antibodies and nuclear dye Hoechst, thus achieving random images with fluorescence microscope. Hence, through three different experiments: cell cycle and cellular growth, dopamine immunoreactivity and neurite density differentiation alterations induced by the presence of mycotoxins will be investigated.

Acknowledgements: *Spanish Ministry of Science and Innovation Project (PID2019-108070RB-I00-ALI) and PhD grant (BES-2017-081328).*

Keywords: SH-SY5Y, Flow cytometry, Microscopy, Retinoic acid, Differentiation.

STUDY OF ENZYMATIC DEFENSE SYSTEM IN NEUROBLASTOMA CELLS EXPOSED TO ZEARALENONE'S METABOLITES AND BEAUVERICIN

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Beauvericin (BEA), α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), are produced by several *Fusarium* species that contaminate cereal grains. These mycotoxins can cause cytotoxicity and genotoxicity in various cell lines and they are also capable of produce oxidative stress at molecular level. However, mammalian cells are equipped with a protective endogenous antioxidant system formed by no-enzymatic antioxidant and enzymatic protective systems such as glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT).

The aim of this study was evaluating the effects of α-ZEL, β-ZEL and BEA, on enzymatic GPx, GST, SOD and CAT activity in human neuroblastoma cells using the SH-SY5Y cell line, over 24h and 48h with individual treatment at the concentration range from 1.56 to 12.5 μM for α-ZEL and β-ZEL, from 0.39 to 2.5 μM for BEA, from 1.87 to 25 μM for binary combinations and from 3.43 to 27.5 μM for tertiary combination.

Our results revealed a significant increase in GPx activity, after 24 h of exposure in all treatments except for tertiary combination which decreased notably; while after 48h, only BEA and triple mixture increased GPx activity considerably. GST activity in SH-SY5Y cells decreased significantly after exposing them to α-ZEL and β-ZEL, while in combinations increased notably after 24h, except for β-ZEL + BEA which a considerable decrease at lowest concentrations and increase at highest concentrations was detected. After 48h, a significant increase in β-ZEL and BEA, whereas a decrease in α-ZEL + β-ZEL combination was observed. CAT activity decreased significantly in all treatments after 24 h except in β-ZEL + BEA, which revealed an increase. For SOD, it was not observed any change after 24 h, although a significant increase was observed in binary combinations α-ZEL + BEA and β-ZEL + BEA after 48h.

Keywords: enzymatic defense; SH-SY5Y cells; zearalenone derivates; beauvericin

PROTEOMIC CHANGES ASSOCIATED WITH ENNIATINS ACUTE EXPOSURE IN RAT LIVER

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Enniatins (ENs) are hexadepsipeptides produced by *Fusarium* fungi which can act as ionophores, disturbing membranes homeostasis. In this study, a proteomic analysis to determine acute response of rat's liver to ENs exposure at different concentrations was carried out. A total of 14 female two months old Wistar rats were employed divided in three groups. Five of the treated ones were intoxicated with medium concentrations: single dose of EN A 256, ENA1 353, ENB 540, ENB1 296 μg/mL; and other five with the higher ones: single dose of ENA 513, ENA1 706, ENB 1021, ENB1 593 μg/mL during 8 hours exposure. Protein extraction was performed using 10 mg of powdered liver tissue in an 8M Urea/2M Thiourea/50mM Tris-HCl lysis buffer. Protein concentration was determined by using a spectrophotometer NanoDrop™ 2000 and subsequently standardized to 1 mg/mL. Samples were mixed with dithiotreitol and iodoacetamide for alkylation of cysteine residues and digested with addition of trypsin (1:40) overnight. Peptides were dried on a vacuum concentrator and eluted in 0.1 % acetic acid: acetonitrile (98:2 v/v) to a final concentration of 100 μg/μL. Samples were analysed using a LC system coupled with quadrupole time of flight (Q-TOF) and the obtained chromatograms were aligned with Mass Hunter Professional software (Agilent). Peptides identification was carried out by Spectrum Mill software and statistically filtered by abundance using Mass Professional Profiler software (Agilent). Results reported a total of 57 differentially expressed proteins in both medium and high treated animals when compared to the control. DAVID gene ontology analysis revealed acetylation, nucleotide phosphate-binding region:NAD and catalytic activity as the most represented terms in the bioinformatics analysis. Moreover, 13 of these proteins were found in the mitochondrion and 12 were related to oxidoreductase activity. Regarding Reactome overrepresentation test results, metabolism was both the most significant pathway and the most enriched.

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Keywords: mycotoxin, proteomics, Q-TOF, mitochondrion, metabolism.

Posters

IN VITRO METHOD DEVELOPMENT FOR THE ASSESSMENT OF OCHRATOXIN AND AFLATOXINS NEUROTOXICITY AND MITIGATION STRATEGIES WITH CAROTENOIDS.

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Aflatoxins and ochratoxin-neurotoxicity is a field of interest for food science researchers as they are able to cross the blood brain barrier triggering mechanisms such as altered gene expression and oxidative stress which are related to neurodegenerative disorders. On the contrary, carotenoids are known for their antioxidant capacity and they have also been found in the brain. So, a method using SH-SY5Y neuroblastoma cells and low concentrations of mycotoxins (100 nM) and carotenoids (500 nM) is being developed simulating a real scenario in the human body. First, the effect of these mycotoxins on SH-SY5Y differentiation, individually and combined, will be assessed through optical, immunofluorescence and confocal microscopy as well as flow cytometry. Optical microscopy will be used to visually discriminate non-differentiated cells, due to diverse morphology. Immunofluorescence will allow to estimate neurite density through β-III tubulin detection, a marker for neuronal differentiation. Confocal microscopy will serve to detect dopamine immunoreactivity, which is also characteristic of neuronal cells activity. Second, Next Generation Sequencing (NGS) will be performed to find the most altered genes by the different cell exposures on an Illumina sequencer by RNA-seq technique. Data analysis will be performed on different bioinformatics tools for Differential Gene Expression (DEGs) and altered pathways. Third, validation of NGS will be done on the most affected genes by qPCR to confirm DEGs. Fourth, proteomics will be carried out in order to analyze if the profile is also modified and these results will be compared with DEGs. Mass spectrometry technique (LC/Q-TOF MS), will allow to quantify the proteome followed by bioinformatics for peptide, protein identification and pathways involved.

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Keywords: SH-SY5Y, ochratoxin a, aflatoxin, neurodegenerative disorders, omics

BIOMONITORING STUDY OF CITRININ AND ITS METABOLITE DIHYDROCITRINONE IN HUMAN URINE

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Citrinin is a mycotoxin produced by *Penicillium* and *Aspergillus* spp. with nephrotoxicity and genotoxicity attributed in human cell lines. Therefore, the European Food Safety Authority set a threshold of toxicological concern for citrinin at 0.2 µg/kg b.w., but the lack of data on food contamination hampers a proper exposure assessment. To overcome this, strategies based on the biomonitoring of citrinin in biological samples have become an alternative in order to assess the human exposure. Since the toxins occur at very low concentrations in human samples, selective and sensitive methodologies are required for accurate measurements. Hence, the aim of this study was to evaluate the presence of citrinin and its metabolite dihydrocitrinone for the first time in 300 human urine samples from South Italy through an ultrahigh-performance liquid chromatography high resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS) methodology. Citrinin was quantified in 47% (*n = 300*) of samples with concentrations ranging from below the limit of quantification (LOQ = 0.012 ng/mL) to 4.003 ng/mL (mean value = 0.286 ng/mL), and dihydrocitrinone was detected in 21% of samples at levels from below the LOQ (0.012 ng/mL) up to 2.481 ng/mL (mean value = 0.386 ng/mL). These results are in accordance with previous works where the metabolite reflects higher average levels than the parental compound. A heavier contamination was observed when compared to other European countries, with a six-to-ten-fold increase of mean values, that could be explained by a higher intake of cereals within the Italian population. Statistical analysis revealed differences according to the age of the volunteers, with citrinin being significantly more present in population from 30 to 60 years old that may be due to different dietary habits. These data reflect a high exposure to citrinin within Italian population, supporting further toxicological and food safety investigations for a better understanding of its impact.

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Keywords: Citrinin; exposure assessment; biomonitoring; urine; Orbitrap

HOW CLIMATE CHANGE IS AFFECTING TO THE OCCURRENCE OF MYCOTOXINS IN EUROPE?

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The impact of climate change on the presence of mycotoxins in food and feed is a topic of great concern. Mycotoxins are ubiquitously present in feeding stuffs, being to date, *Fusarium* mycotoxins (deoxynivalenol, DON; zearalenone, ZEN; and fumonisins, FBs) prevalent in areas of temperate climates such as Europe and *Aspergillus* mycotoxins contamination more frequently in hot climates. Every mould species has its own optimum conditions of temperature and a_w for growth and formation of mycotoxins, so environmental factors such as high temperatures, high moisture levels, and insect damage contribute to the presence of mycotoxins in feeds. The aim of this work was to review recent data reported on the mycotoxins occurrence patterns in Europe because of environmental changes.

In the data obtained from the available literature, the evidence shows *A. flavus* infection and AFs contamination, previously uncommon in Europe, would become increasingly important. Over the last decade, several hot seasons have led to severe *A. flavus* infections in maize in several European countries (Italy, Romania, Serbia and Spain). Aflatoxin (AF) outbreaks have been reported in some regions of South of Europe, such as infection of maize by AFB1 in Italy from 2003 as result of a hot and dry growing season. It is even being detected AFM1 in cow's milk samples, in which differences depending on the season of sampling have been observed.

On the other hand, the prevalence of *Fusarium graminearum* (specie adapted to hot conditions) and the main productor of DON in cereal grain, has already increased in Central Europe and is likely to increase in the North Europe due to the expected changes in weather conditions.

These facts point out that occurrence patterns of mycotoxins in Europe are changing as a consequence of rising average temperatures and consequently a potential increase of consumer health risk in Europe.

Acknowledgements: *Authors are grateful to Regional Government to fund GV/2020/020 project*

Keywords: mycotoxins, climate change, occurrence patterns, Europe

EVALUATION OF MYCOTOXINS IN INFANT BREAST MILK

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A recent review of presence of mycotoxins in breast milk published in the last years is presented. It has been carried out to provide an overview of infant population exposure by countries and continents. OTA and AFM1 were the most present. The global mean and range detected was greatly different between continents and countries. It was observed a high incidence in Tanzania, Iran, Jordan and Turkey, however the highest values were observed in Egypt, Sudan and Serbia, being higher than the EU maximum limits (25 ng/kg for AFM1 and 500 ng/kg for OTA) (Regulation (EC) No 1881/2006). It was calculated a provisional estimated daily intake (PDI), using the mean observed and an approximation of daily consumption of breast milk from 630 g/day to 890 g/day. EFSA (2006) established a tolerable daily intake (TDI) only for OTA with 14 ng/kg/day and non-intake for AFM1. It should be noted that the AFM1 values ranged from <1 ng/L to 7100 ng/L (Brazil and Egypt, respectively), then PDI were from 0.5 and 595 ng/kg bw/day. On the other hand, the range of OTA´s means were 4-1990 ng/L (Brazil and Iran) with an PDI from 0.38 to117 ng/kg bw/day, values that represent the 3-1194% of the TDI. The review indicates that more controls on raw materials should be applied in Egypt, Iran and Turkey.

Keywords: mycotoxin, breast milk, infant, aflatoxin, ochratoxin A

INHIBITION OF *Fusarium verticillioides* IN TOMATO FRUITS BY EXTRACTS OF *Trichoderma* spp.

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Tomato fruit rot is a serious disease caused by *Fusarium* species. The aim of this study is to evaluate the ability of two strains of *Trichoderma* (*T. asperellum* and *T. atroviride*) to inhibit *Fusarium* spp.
The inhibitory effect of *Trichoderma* extract with ethylacetate (EtOAc) was studied *in vitro* and *in vivo*. *Trichoderma* was grown in PDB at 30°C for 30 days. The cultured filtrate was extracted with EtOAc. The extract of *T. asperellum* (TaE) and *T. atroviride* (TatE) were tested *in vitro* against six species of *Fusarium* (in 96-well plates) obtaining the minimum inhibitory concentration and the minimum fungicidal concentration (MIC & MFC).
Starting from MFC, three concentrations were tested *in vivo* on tomato fruits. Tomato fruits were inoculated with *F. verticillioides*, treated with the extracts at three different concentrations (ranges 0.78-3.12 mg/ml) and incubated at 4 and 11 days to room temperature.
The extracts showed MIC values ranging between 0.19 and 0.78 and MFC values ranging between 0.78 and 1.56 mg/ml for TaE and TatE, respectively.
In vivo, both extracts (at 3.12 mg/ml) showed efficacy in inhibiting the growth of *F. verticillioides* with significant difference ($p<0.05$) compared to control (untreated). At 4 days after treatment, infection rates (IR%) of 7.5% and 23%, and Log CFU/g of 2.9 and 5.2 were for TaE and TatE, respectively. However, only TatE showed persistent efficacy at 11 days with an IR% of 19% and 4.8 Log CFU/g.

Keywords: antifungal activity, *Trichoderma asperellum*, *Trichoderma atroviride*, tomato fruit rot

CHARACTERIZATION OF THE ANTIFUNGAL POTENTIAL OF LACTIC ACID BACTERIA ISOLATED FROM RED GRAPE

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Filamentous fungi infection is the principal cause of fruit and vegetal losses in the field. Chemical pesticides are the regular response to those infections. Nevertheless, the exaggerated use of these pesticides as a normal response those these contaminations brought several environmental and health problems. New and safer methods are being tested, among them, the use of microorganisms (MO) as biopreservative strategies to become an alternative to regular pesticides. In this study the antifungal potential of 33 MO isolated from red grape was studied. First, a gram stain was performed to identify the MO. Then, the characterization of the antifungal activity was performed using the agar diffusion method and the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) method, against fungi from *Aspergillus*, *Alternaria*, *Botrytis*, *Fusarium* and *Penicillium* genera. The antifungal agent tested was a cell free supernatant (CFS) from MRS medium fermented by the MO. Gram stain revealed that the MO were yeast and gram-positive coccus bacteria. Agar diffusion method showed that overall, the bacterial CFS was more active against the fungi than the CFS made by yeast. The CFS fermented by UTA6 bacteria exhibited the greatest fungal inhibition. The MIC-MFC exposed similar results, CFS fermented by bacteria had higher antifungal activity, especially UTA6 reaching MIC's from 6.3 to 50 g/L and MFC's from 6.3 to 100 g/L. Future investigations will focus on the identification and quantification of the compounds with antifungal activity from the CFS and the possible application as biopreservative on red grape.

Keywords: Lactic acid bacteria, food contamination, antifungal activity, fungi.

TOXICOLOGICAL INTERACTIONS BETWEEN THE MYCOTOXIN T-2 TOXIN AND ITS MODIFIED FORMS IN HEPG2 CELLS AND PREDICTION OF TOXICITY BY *IN SILICO* APPROACHES

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The T-2 toxin (T-2) is commonly metabolized to HT-2 toxin (HT-2), Neosolaniol (NEO), T2-triol and T2-tetraol and they can modify the toxicity of T-2. In this study, T-2 and its modified forms were evaluated by *in vitro* and *in silico* methods. The *in vitro* cytotoxicity individually was evaluated by MTT and Total Protein Content (PC) assays in human hepatocarcinoma (HepG2) cells. The concentrations tested were from 12.5 to 100 nM (T-2), 18.75 to 150 nM (HT-2), from 11 to 164 nM (NEO), from 164 to 2620 nM (T2-triol) and from 209 to 3350 nM (T-2 tetraol). The order of IC_{50} was T-2 tetraol>T-2 triol>NEO>T-2=HT-2. The T-2 and HT-2 evidenced the highest cytotoxic effect in HepG2 cells. Cytotoxicity of binary mycotoxins combination was evaluated at ratios 1:1 (T-2+HT-2), 1:16 (T-2+T-2 triol and HT-2+T-2 triol), 1:1.4 (T-2+NEO and HT-2+NEO), 1:2.2 (T-2 triol+T-2 tetraol), 1:11.8 (NEO+T-2 triol), 1:26.1 (NEO+T-2 tetraol) and 1:35.4 (T-2+T-2 tetraol and HT-2+T-2 tetraol). All binary combinations exhibited antagonistic interactions. The ADME and toxicity profile of mycotoxins were obtained bythe *in silico* admetSAR predictive model which determines the approaches in order to know if these mycotoxins might be taken into consideration to support a more realistic and adequate risk assessment.

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Keywords: T-2; cytotoxicity; metabolites; interaction, *in silico*

EPIGENETIC CHANGES STUDY IN RAT OVARIES AFTER SUBCHRONIC ORAL EXPOSURE TO ENNIATIN A

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Enniatins are secondary fungal metabolites and worldwide natural contaminants of several food and feed products. A 28-day repeated dose preliminary assay, using enniatin A naturally contaminated feed through microbial fermentation by a *Fusarium tricinctum* strain, was carried out employing 2-month-old female Wistar rats. In order to simulate a physiological test of a toxic compound naturally produced by fungi, five treated animals were fed during 28 days with fermented feed. As control group, five rats were fed with standard feed. Estimated amount of enniatin A in serum were: 22.43, 29.02 and 36.80 μg on the second, third and fourth week, respectively; and enniatin A blood concentrations obtained were: 0.97, 1.25 and 2.70 μg/ml on the second, third and fourth week, respectively. Previous results revealed that the relative number of lymphocytes T cytotoxic cells in the treated rats was inhibited significantly respect to the control ones ($p<0.001$), while lymphocytes T helper cells increased significantly ($p<0.001$). In this study the epigenetic alterations are evaluated in the ovaries of the treated and control rats described, in particular relative telomere length and mitochondrial DNA copy number by real-time PCR and DNA methylation by pyrosequencing. Results regarding telomere length were 1040.29 (1375.40-637.06) for enniatin A exposed rats and 1212.23 (1596.76-674.74) for control ($p=0.55$). The mitochondrial DNA copy number showed a media of 303.73 (611.99-20.86) for treated rats and 501.97 (952.01-66.92) for control ($p=0.51$). Results from DNA methylation by pyrosequencing are still being processed. Even if non-significant results were obtained, a decreasing tendency in mitochondrial DNA copy number and telomere length was observed, so in the future a larger number of samples is recommended to be analysed in order to obtain more concluding results.

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Keywords: epigenetics, mycotoxin, relative telomeres length, mitochondrial DNA copy number, pyrosequencing.

THE MAIN ANIMAL SOURCE FOODS CONTAMINATED BY MYCOTOXINS

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Mycotoxins are common contaminants in raw materials and feedstuffs intended for livestock. Thus, when animals are fed contaminated feeds, the mycotoxin carry-over into animal organs, edible tissues, and by-products (milk, eggs…) can occur. From the public health standpoint regarding mycotoxin occurrence in animal source foods (ASF), the mycotoxins considered to be of the outermost importance are aflatoxin M1 (AFM1) in milk and Ochratoxin A (OTA) in meat products, both classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC). Maximum Levels (MLs) for AFM1 in milk have been set by European Commission (0.05 µg/kg). However, no MLs have been set in Europe for OTA in meat or meat by-products. Notwithstanding, some countries have enforced MLs of OTA concentrations and other countries have developed national guidelines for OTA levels. The aim of this work was to review recent data reported on the AFM1 occurrence in milk and OTA in meat by-products. On the one hand, AFM1 has been widely assayed in milk samples, exhibiting differences depending on the animal species and the season of sampling, showing high contents in cow milk collected during winter season and levels reaching up to 4.2 µg/kg, exceeding the MLs, have been reported. On the other hand, regarding OTA, different surveys reported their occurrence especially in dry-cured meat products made from pork tissues, mainly sausages, ham and different types of salami and prosciutto. High incidence and contents have been reported ranging from 0.06 to 14.7 µg/kg, although high values (up to 691 µg/kg) have been detected in salami samples This fact highlight the need to establish a ML for OTA in these products to protect human health and to constantly monitoring mycotoxin occurrence in animal by-products.

Acknowledgement: *GV/2020/020*

Keywords: mycotoxins; Aflatoxin M1; Ochratoxin A; milk; meat.

OCCURRENCE OF MYCOTOXIGENIC MOLDS IN CITRUS FRUITS OF THE MEDITERRANEAN AREA AND BIOPRESERVATIVE SOLUTIONS

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Citrus are the most important fruit crop in terms of value worldwide. In 2016, the FAO (2017) estimated the world's citrus production at about 124. 2 million tons, with orange accounting for around 67 million tons. Globally about 27 million tons of citrus fruits are transformed industrially, mostly for the production of juice. Quality standard, health of the consumers and a long shelf-life are fundamental aspects affecting the competitiveness of citrus fruits produced by Mediterranean countries on both domestic and international markets. Rots caused by fungi are the main cause of post-harvest losses (estimated average of 30%) of citrus fruits and may consistently reduce their shelf life. Some fungal infections not always causes direct damage or visible symptoms, and some citrus fungal pathogens produces toxins that could pass into the endocarp and contaminates juices too. In this study the fungal occurrence and mycotoxin presence in citrus fruits was studied. In parallel, it was been investigated the presence of lactic acid bacteria from the natural microbiota of citrus fruits as biocontrol solution to prevent the fungal presence in peel and juice, reducing post-harvest rots and mycotoxins contents, respectively. Finally, the isolated bacteria will be tested against filamentous fungi *in vitro* assay to select candidates to biopreservative agents production from orange by-products and its applications like polymeric antifungal films or coatings.

Keywords: citrus fruits, mycotoxigenic fungi, lactic acid bacteria, by-products, biopreservation.

EMERGING TECHNOLOGIES TO MITIGATE AFLATOXIN B2 IN GRAPE JUICE

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Nowadays, people are seeking freshness, high vitamin content, minerals and low-calorie products (Mandappa et al., 2018). This fact encouraged the implementation of non-thermal food processing techniques, such as high pressure processing (HPP) and pulsed electric fields (PEF), with low impact on food nutritional components (Picart-Palmade et al., 2018). These technologies are explored for mycotoxin reduction or elimination without producing toxic residues (Gavahian et al., 2020). Aflatoxins (AFs) constitute one of the most investigated mycotoxins and were reported in food commodities such as groundnuts, sesame seeds, millet, maize, rice, wheat, fig, spices, and cocoa (Mahato et al., 2019). AFs are carcinogenic, mutagenic and immuno-suppressive compounds, that have been correlated with liver cancer (Marín et al., 2013). The European Commission (EC) has set maximal concentrations of AFB1 and the sum of AFB1, AFB2, AFG1 and AFG2 in certain foodstuffs, but not maximum levels of AFs have been set in juices (EC, 2006). The aim of this study is to investigate the effect of HPP and PEF technologies on AFB2 reduction in grape juice. Grape juice samples were spiked with AFB2 at concentration of 100 μg/L and treated by HPP and PEF technologies. PEF treatment was carried out under conditions of field strength of 3 Kv /cm and specific energy of 500 KJ/kg. HPP treatment conditions were set in pressure at 500 MPa during 5 minutes. After both treatments, AFB2 was extracted employing dispersive liquid-liquid microextraction method (DLLME) and determined by liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/ MS-IT). The reductions observed were about 72% after PEF treatment and 14% after HPP. Moreover, an AFB2 degradation product with m/z 355.0711 has been identified by quadrupole time of flight mass spectrometry detector (qTOF-MS) after PEF treatment.

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Keywords: aflatoxin B2; HPP; PEF; decontamination; grape juice

PREDICTING TOXICITY AND METABOLOMIC PROFILE *IN SILICO* FOR ZEARALENONE, α-ZEARALENOL AND β-ZEARALENOL

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Mycotoxins are present in stored grain and co-exposure through the diet is very common. Although several studies have been done *in vitro* and *in vivo*, the toxic effects associated to metabolites generated once ingested are still unknown and difficult to study. The present study defines the metabolomics profile of all three mycotoxins (zearalenone (ZEA), α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL)) and explores the prediction of their toxic effects proposing an *in silico* workflow by using three programs of predictions: MetaTox, SwissADME and PASS online. Metabolomic profile was also defined and toxic effect evaluated for all metabolite products from Phase I and II reaction (a total of 15 compounds).

Results revealed that products describing metabolomics profile were: from O-glucuronidation (1z and 2z for ZEA and 1ab, 2ab and 3ab for ZEA´s metabolites), S-sulfation (3z and 4z for ZEA and 4ab, 5ab and 6ab for ZEA´s metabolites) and hydrolysis (5z and 7ab for ZEA´s metabolites, respectively). Lipinsky´s rule-of-five was followed by all compounds except those coming from O-glucuronidation (HBA>10). Metabolite products had better properties to reach blood brain barrier than initial mycotoxins. According to Pa values (probability of activation) order of toxic effects studied was carcinogenicity > nephrotoxic > hepatotoxic > endocrine disruptor > mutagenic (AMES TEST) > genotoxic. Prediction of inhibition, induction and substrate function on different isoforms of Cytochrome P450 (CYP1A1, CYP1A2, CYP2C9 and CYP3A4) varied for each compounds analyzed; similarly, for activation of caspases 3 and 8. Relying to our findings, the metabolomics profile of ZEA, α-ZEL and β-ZEL analyzed by *in silico* programs predicts alteration of systems/pathways/mechanisms that ends up causing several toxic effects, giving an excellent sight and direct studies before starting *in vitro* or *in vivo* assays contributing to 3Rs principle; however, confirmation can be only demonstrated by performing those assays.

Keywords: Zearalenone; Metabolomics; Prediction; SwissADME; PASS online; MetaTox; In silico

SCREENING OF LACTIC ACID BACTERIA WITH ANTIFUNGAL ACTIVITY ISOLATED FROM DRY-CURED MEATS

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Fungal spoilage is not only a global food quality concern but also presents serious health problems due to the production of mycotoxins, some of which present considerable challenges to food safety and generate large economic losses. Chemical preservatives are successful in retarding microbial growth, yet the growing demand for clean label products requires manufacturers to find natural alternatives to replace chemical ingredients. Lactic acid bacteria (LAB) are generally recognized as safe (GRAS), therefore they are considered a good candidate for their use as a natural preservative in food to control fungal growth and subsequent mycotoxin production, as well as to improve shelf life. The objectives of this study were to identify and characterize LAB isolated from dry-cured meats with the potential to inhibit the growth of five toxigenic fungi (*Aspergillus flavus* ITEM 8111, *Cladosporium oxysporum* CECT 20421, *Penicillium nordicum* CECT 2320, *Penicillium verrucosum* VTT 47, and *Penicillium griseofulvum* CECT 2605). The *A. flavus* proved to be more resistant against all the LAB strains tested. From a total of ninety LAB strains isolated, seven were selected for their high growth inhibitory effect in direct contact with the fungus by overlay technique, and another seven were selected for their capacity to inhibit fungal growth by halo diffusion assay. In addition, the ability of the bacteria to hydrolyze meat proteins was also considered. For further studies, we propose the application of fermented swine loin extracts produced by these bacteria in casings to manufacture dry-cured meat products.

Keywords: Lactic Acid Bacteria; Mycotoxins; Dry-cured meat; Antifungal; Fungal spoilage.

APPLICATION OF THE YELLOW MUSTARD BRAN IN BREAD AS A NATURAL PRESERVATIVE AGENT

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Fungal spoilage causes important economic losses in the food industry and is a challenge in food safety due the production of mycotoxins, highly toxic compounds. In the past years multiple strategies have been evaluated using natural compounds to prevent fungal spoilage. The aim of the study was to evaluate the antifungal properties of Yellow Mustard Seed (YMS) and Yellow Mustard Bran (YMB) extracts against toxigenic fungi of the *Aspergillus*, *Penicillium* and *Fusarium* genera. For this, a qualitative evaluation test on PDA plates was performed. YMB evidenced the highest antifungal activity and for this reason, the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were established *in vitro*. The *Penicillium* genera evidenced the lowest MIC and MFC values, ranging from 0.3 to 4.7 g/L. Then, the use of YMB was studied to increase the shelf life of bread contaminated with *P. commune* CECT 20767. For this, different amounts of YMB were tested (2.5, 5, 7.5 and 10 g/Kg). In addition, a commercial treatment with sodium propionate (E-281) and a control treatment without preservatives were performed. The use of 10 g/Kg increased the shelf life in comparison to the control by 3 days and, in addition, equated the reduction of fungal population as the commercial treatment. These results suggest the promising employment of YMB as an antifungal additive in the food industry because satisfy the consumers' demand for natural additives.

Keywords: Yellow Mustard, Antifungal activity, *Penicillium*, Bread

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Mesa redonda Grupos de investigación-Empresas

Importancia de la Seguridad Alimentaria en la industria post covid-19.

FOOD PACKAGING CHALLENGE IN THE AGE OF COVID-19

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The food packaging industry is facing a series of challenges as consumers and the rest of the world deal with changes related to COVID-19.

Customer demand has shifted drastically, pandemic shat down restaurants and food-service outlets. Consumers have moved to buy at grocery purchases, for which the use of packaging has risen.

Consumers' wishes to stockpile, and their panic purchases of food, beverages, and home-care necessities have accentuated this trend.

Single-use, disposable food packaging appears to have made a comeback rising on the coattails of the COVID-19 pandemic, as many consumers believe this to be safer and/or more hygienic, but is this true?

In food packaging challenge in the age of COVID 19 presentation we will review which are the food packaging challenge and how the sector can solve them.

Keywords: food packaging, active, edible.

COVID 19: IT IS NOT A FOOD CRISIS, BUT IT IS AN IMPORTANT CHALLENGE FOR THE FOOD INDUSTRY

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COVID-19 is not considered a food transmission disease. The European Food Safety Agency (EFSA) is closely monitoring the information generated in the context of this crisis and has reported that there is no evidence that food is a COVID-19 source or route. The same view has been expressed by the FDA (Food and Drug Administration of the United States) and WHO.

However, food sector has a triple challenge in this pandemic, meeting unusual demand, safeguarding workers health and ensuring food safety of products. To achieve this triple objective (focusing on measures that have an impact on food safety and worker health) it is important to strengthen hygiene practices in processing and handling operations. Food sector currently has robust food safety management systems, like APPCC (hazard analysis and critical control points) including the adoption of correct hygiene practices in mandatory form or BRC, IFS and ISO 22.000 as non-mandatory systems.

We can therefore ensure that food sector has the necessary tools to ensure food safety, but it is important to take measures to strengthen food hygiene practices and their monitoring.

At the first weeks of the pandemic, we considered that could be useful to develop the "COVID Manual. Strengthening food hygiene measures in the productive environment" in order to provide food industries with guidelines to deal with this special situation.

This manual includes measures concerning plant staff, visits and other operations, such as maintenance, cleaning and disinfection, etc. It has received more than 3,000 downloads and has been presented in two webinars with more than 1,000 attendees.

MICOTOXIN RISK MANAGEMENT IN FOOD INDUSTRY

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Nuts are suitable substrates for fungal growth if production and trade conditions are appropriate. It is well-known that some of these moulds can be mycotoxigenic, thus several mycotoxins may be present in our products. According to Food Agriculture Organization (FAO) around 25% of crops are affected by mycotoxigenic moulds worldwide and, it has been estimated that over 1000 million tons are lost each year due to this reason. Besides, according to the annual report of the Rapid Alert System for Food and Feed, nuts were two of the most affected food categories by mycotoxins. Aflatoxins were the primary mycotoxins associated to the notifications, but more and more, other types and emergent mycotoxins also of relative concern for food industry. Therefore, this is one of the main concerns in our enterprise.

Traditionally, food safety control at food industry consisted in determining the levels of mycotoxins in acquired goods according to legislated levels. This is one-side control mechanism that does not provide a complete information considering the heterogeneous distribution of micotoxins. However, in our enterprise we designed a 360º model based on a multifactorial point of view. This model is intended to ensure food safety and quality control throughout the entire food production chain, namely from farm to fork. This way we are capable of gathering many data from several key points that enable us to work in a preventive approach instead of a corrective mode. Our final aim is to achieve a predictive model that guarantees no presence of mycotoxins in our goods from the very first beginning of the chain.

Keywords: micotoxin, nuts, predictive quality, multifactorial model

Colaboran

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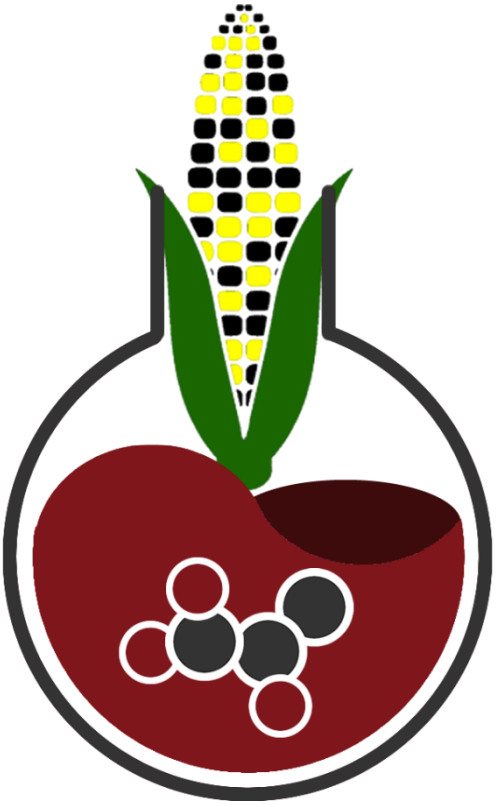

Estrategias biotecnológicas para reducir el crecimiento de hongos toxigénicos en alimentos.

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