# CONTAMINATED MILKS AFTER *IN VITRO* HUMAN DIGESTION

Tania Aparecida Becker-Algeri [1,2,6], Carina Ladeira[2,3,4], Andreia Isabel Pimenta [5], Sandra Cabo Verde [5], Susana Viegas[2,4], Deisy Alessandra Drunkler[6]; Eliana Badiale-Furlong[1]

[1]Department of Food Chemistry, Federal University of Rio Grande, Rio Grande, Brazil; [2]Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa, ESTeSL, Instituto Politécnico de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal; [4]Centro de Investigação em Saúde Pública, Escola Nacional de Saúde Pública, Lisbon, Portugal; [5] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal; [6]Universidade Tecnologica Federal do Paraná, Medianeira, Brasil.

## Introduction

ilk is considered a complete food from the nutritional point of view. Milk can be exposed to various types of contaminatio such as mycotoxins. These metabolites are naturally occurring toxic compounds produced by fungi. Several studies on milk amples have reported the presence of aflatoxin $B_1$ ($AFB_1$) and $M_1$ ($AFM_1$), due to the high incidence in samples intended fo man consumption, carcinogenicity proven $AFB_1$ and resistance of the contaminants to the process of digestion, making tho ilable for intestinal absorption. Considering these aspects, the objective of this study was to evaluate the genotoxicity of m mples contaminated by $AFB_1$ and $AFM_1$ before and after the action of lactic acid bacteria using Caco-2 intestinal human ce

## Material and methods

### *Sample preparation*

Homogenized pasteurized milk

Spiked with $AFM_1$ our/and $AFB_1$ (2 µg.L$^{-1}$ and 10 µg.L$^{-1}$)

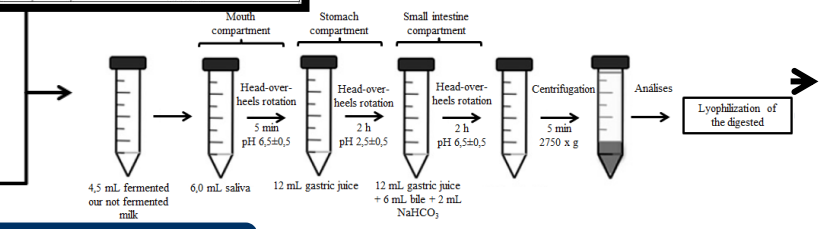
Inoculation of LAB (08 assay + Control + Positive Controls)

Collect time 0h incubation → *In vitro* digestion

Incubation (42°C until pH 4,4 - 4,9)

Collect final time (pH 4,4- 4,9) → *In vitro* digestion

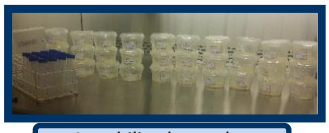
| Assay | Aflatoxin | LAB |
|---|---|---|
| Control | - | - |
| PC | $M_1$+$B_1$ | - |
| 1 | $M_1$+$B_1$ | *L. delbrueckii* subs. *Bulgaricus* |
| 2 | $M_1$+$B_1$ | *L. acidophilus* |
| 3 | $M_1$+$B_1$ | *B. lactis* HN019 |
| 4 | $M_1$+$B_1$ | *S. salivarius* ssp. *thermophilus* |
| 5 | $M_1$+$B_1$ | *L. delbrueckii* subs. *Bulgaricus* + *S. salivarius* ssp. *thermophilus* |
| 6 | $M_1$+$B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* |
| 7 | $M_1$+$B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *B. lactis* HN019 |
| 8 | $M_1$+$B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* + *B. lactis* HN019 |
| Controle | - | - |
| PC $M_1$ | $M_1$ | - |
| 1 | $M_1$ | *L. delbrueckii* subs. *Bulgaricus* |
| 2 | $M_1$ | *L. acidophilus* |
| 3 | $M_1$ | *B. lactis* HN019 |
| 4 | $M_1$ | *S. salivarius* ssp. *thermophilus* |
| 5 | $M_1$ | *L. delbrueckii* subs. *Bulgaricus* + *S. salivarius* ssp. *thermophilus* |
| 6 | $M_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* |
| 7 | $M_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *B. lactis* HN019 |
| 8 | $M_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* + *B. lactis* HN019 |
| PC $B_1$ | $B_1$ | - |
| 1 | $B_1$ | *L. delbrueckii* subs. *bulgaricus* |
| 2 | $B_1$ | *L. acidophilus* |
| 3 | $B_1$ | *B. lactis* HN019 |
| 4 | $B_1$ | *S. salivarius* ssp. *thermophilus* |
| 5 | $B_1$ | *L. delbrueckii* subs. *Bulgaricus* + *S. salivarius* ssp. *thermophilus* |
| 6 | $B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* |
| 7 | $B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *B. lactis* HN019 |
| 8 | $B_1$ | *L. delbrueckii* subs. *bulgaricus* + *S. salivarius* ssp. *thermophilus* + *L. acidophilus* + *B. lactis* HN019 |




The pasteurized milk samples were spiked with $AFB_1$ (10 µg.mL$^{-1}$) $AFM_1$ (2 µg.mL$^{-1}$) and subjected to fermentation with 4 differe lactic acid bacteria (LAB) (*Lactobacillus delbrueckii* subs. *bulgaric acidophilus*, *Bifidobacterium lactis* HN019 and *Streptococcu salivarius* ssp. *thermophilus*) in separate and in combined form totaling twenty four fermentation tests beyond the Positive Co (only milk and mycotoxin) and Negative Control (only milk). T samples were incubated at 37°C and fermented milk products (4 4.9). The samples were digested. The digestion model a model *vitro* digestion based in an initial saliva processing for 5 min at 3 to simulate the mouth compartment and the gastric conditions h, followed by simulated small intestine compartment for 2 h 37°C. The digested samples were lyophilized to use in cell cultu

*Exposure of Caco-2 cell's culture*

### *Exposure of Caco-2 cell*



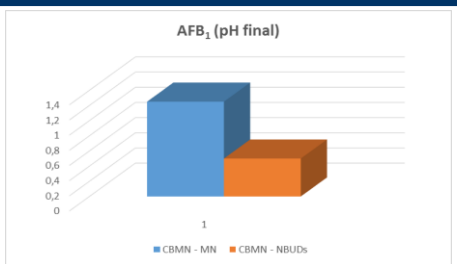
Lyophilized samples

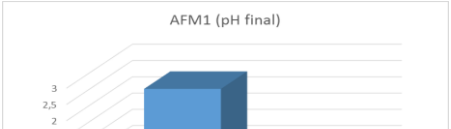
Caco-2 cells (1 x $10^6$ cells/well)

Genotoxicity assessment by the cytokinesis-block micronucleus assay (quantification of micronuclei in *Caco-2 cells)*

Digested samples were reconstituted and prepared for exposure of Caco-2 cells
(100 µL sample in 900 µL of medium and cells) for 24 hours.

## ts and discussion




The $AFB_1$+$AFM_1$ before and after digestion and $AFB_1$ and $AFM_1$ before digestion, d not show cell viability before and after fermentation, and therefore genotoxicity cou not be assessed. $AFB_1$ after digestion induced in exposed Caco-2 cells 1. micronucleus (MN) and 0.5 nuclear buds (NBUDs), and $AFM_1$ exposed cells present 2.57 MN and 0.571 NBUDs. According to this results, $AFM_1$ seems to have mo genotoxic potential in Caco-2 cells in comparison with $AFB_1$, however limitations d to cellular viability did not allow to take more robust conclusions.

The use of lactic acid bacteria in fermentation of milk contaminated with aflatoxins and $M_1$ promoted decreasing genotoxicity of the two mycotoxins, especially wh contaminated with $AFB_1$.