ASSESMENT OF GENOTOXICITY OF AFLATOXIN M_1 AND B_1 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]

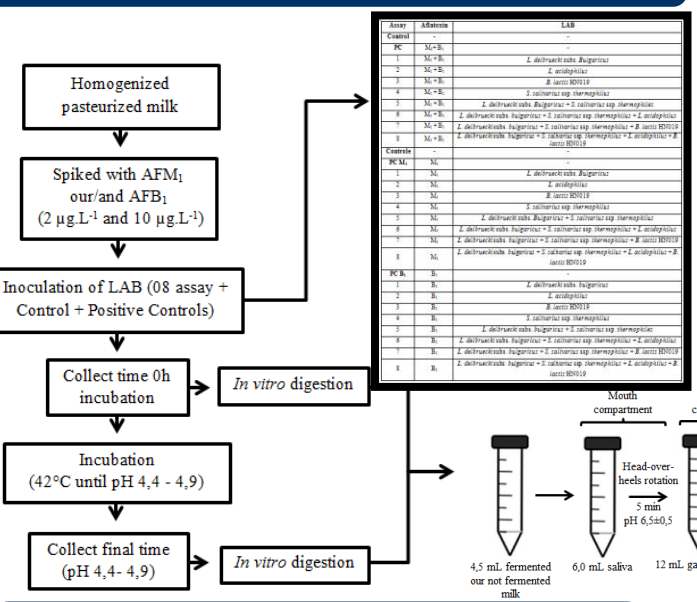
[1]Department of Food Chemistry, Federal University of Rio Grande, Rio Grande, Brazil; [2]Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa, ESTeSL, Instituto Politécnico de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal; [4]Centro de Investigação 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



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
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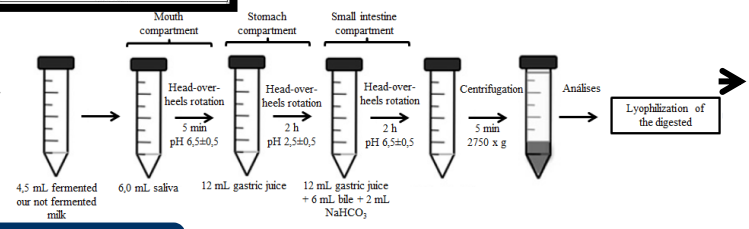
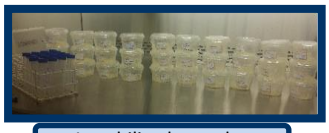
The pasteurized milk samples were spiked with AFB_1 (10 µg.mL^{-1} AFM_1 (2 µg.mL^{-1}) and subjected to fermentation with 4 differe lactic acid bacteria (LAB) (*Lactobacillus delbrueckii* subs. *bulgaric acidophilus*, *Bifidobacterium lactis* HN019 and *Streptococcu salivarius* ssp. *thermophilus*) 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



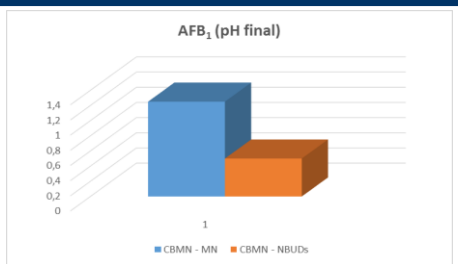
Lyophilized samples

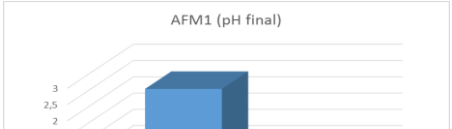
Caco-2 cells (1 x 10^6 cells/well)

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.

ults 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.