Efficiency of different sources of *Saccharomyces cerevisiae* for decontamination of aflatoxin B_1 in Phosphate buffer saline solution

Carlos Humberto Corassin, DVM, Ms, PhD.

carloscorassin@usp.br

Professor Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo - Brazil

Aflatoxins

- Are one of the most important mycotoxins known, being *Aspergillus* species distributed worldwide, with optimal growth conditions: relative humidity of 80-85% and temperature around 30ºC.

- Are secondary metabolites of low molecular weight produced by filamentous fungi, particularly *Aspergillus flavus* and *A. parasiticus*, distinguished by their wide distribution in food and pronounced toxic properties.

Aflatoxins

- Aflatoxins are also of great importance for the Public Health, as they are one of main factors involved in the etiology of human hepatic cancer - hepatocellular carcinoma, as a consequence of the ingestion of contaminated foods.

- This disease represents more than 80% of primary malignant tumors of the liver, and it is the 7th to 9th most common type of cancer worldwide affecting men and women, respectively.

Aflatoxins

- Practical difficulties to effectively prevent contamination, along with the stability of aflatoxins under normal food processing conditions, have led to investigation on decontamination methods for food products.

- The use of microorganisms offers an attractive alternative for the control or elimination of aflatoxins in foodstuffs.

- *Saccharomyces cerevisiae* is the most effective for binding AFB_1

Objectives

- The aim of the present study was to evaluate the ability of a *Saccharomyces cerevisiae* strain from four different sources:
  - Dried yeast from sugar cane yeast (DY)
  - Autolyzed yeast from sugar cane yeast AY)
  - Cell wall from sugar cane yeast (CW)
  - Brewery dehydrated residue (BDR)

- To bind AFB_1 in phosphate buffer saline (PBS) spiked with 0.5 ng mL^{-1} AFB_1, during contact times of 5, 10, 20 and 30 minutes.

Materials and methods

- Commercially available sugar cane yeast (dried yeast - DY, autolyzed yeast - AY, cell wall - CW) and *brewery yeast* (brewery dehydrated residue - BDR).
- The products were weighed to reaching a cell concentration of 1.0×10^{10} cells mL^{-1}.
- All SC cells were heat-killed, being inactivated by autoclaving at 121º C for 10 minutes before the binding assays, to avoid any possible fermentation during the contact time.

Materials and methods

- AFB_1 standard solution was diluted in acetonitrile and spectrophotometrically calibrated in order to obtain a stock solution.

- A working solution was prepared in PBS, pH 7.3, evaporating the acetonitrile until visible acetonitrile droplets disappeared.

- The assay of AFB_1 binding in PBS was performed in triplicate. A volume of SC strains from each different source were transferred to Eppendorf tubes and suspended in PBS spiked with AFB_1.

Materials and methods

- Following the contact times of 5, 10, 20 and 30 min., the tubes were centrifuged for 15 minutes, and the supernatant removed for analysis of AFB_1.

- The same procedures as described above were performed in triplicate positive controls, negative controls and non-spiked PBS controls.

Materials and methods

– Quantification of AFB_1 in PBS solutions was performed by injection of supernatant in a high-performance liquid chromatograph (HPLC) Shimadzu® system (Tokyo, Japan), consisting of a fluorescence detector RF-10A XL (Shimadzu®) equipped with a Synergy Fusion column 4 µm C18 4.6×150 mm (Phenomenex®, Torrance, USA) and autosampler SIL- 10AF (Shimadzu®).

Materials and methods

- Detection was made at an excitation wavelength of 366 nm and emission at 428 nm.

- Detection limit for AFB_1 was 0.01 ng/mL, as considered by the minimum amount of AFB_1 that could generate a chromatographic peak 3 times over the baseline standard deviation. Retention time of AFB_1 was 6.9 min.

- Statistical analysis of AFB_1 binding assays was carried out in the General Linear Model by using the Tukey Test for significant differences between the sources tested and contact time.

Results and discussion

- Lower AFB_1 levels were found in PBS spiked with AFB_1 after treatment with dried yeast from sugar cane (DY) cells, with values ranging from not detected (LOD: 0.01 µg mL^{-1}) to 0.035±0.002 µg mL^{-1}.
- The second best response was achieved using the autolyzed yeast from sugar cane (AY), with AFB_1 remaining in PBS at levels from 0.025±0.006 to 0.096±0.005 µg mL^{-1}.
- Cell wall yeast from sugar cane (CW) and brewery dehydrated yeast residue (BDR) treatments had AFB_1 in PBS at levels ranging from 0.102±0.011 to 0.217±0.009 µg mL^{-1}.

Results and discussion

– Percentages of aflatoxin B_1 bound to *S. cerevisae* products at different contact times in PBS.

S. cerevisae products	% of bound AFB_1 (mean ± SD)			
	5 min.	10 min.	20 min.	30 min.
Dried yeast	99.3 ± 0.2^{a}	97.9 ± 0.5^{a}	97.8 ± 0.8^{a}	96.5 ± 1.1^{a}
Autolyzed yeast	95.3 ± 1.4^{a}	97.5 ± 1.1^{a}	96.6 ± 1.6^{a}	94.9 ± 1.3^{a}
Cell wall	80.1 ± 0.5^{b}	78.3 ± 0.9^{b}	83.6 ± 0.7^{b}	86.1 ± 0.8^{b}
Brewery residue	81.0 ± 0.3^{b}	86.0 ± 0.8^{b}	83.7 ± 0.2^{b}	87.8 ± 0.7^{b}

$^{a-b}$ In the same column, means followed by different superscript letters differ significantly ($p<0.05$).

Results and discussion

- By the findings of this study, it is apparent that celular viability is not a prerequisite for removal of AFB_1 by SC.

- The mechanism involved in SC ability to bind aflatoxins remains unclear. It is currently accepted that yeast cell wall has the ability to adsorb the toxin.

- In the present study, SC cells bound from 99.3 ± 0.2% (using DY for 5 min) to 78.3 ± 0.9% (using CW for 10 min) of AFB_1 content in PBS.

Conclusion

- Heat-killed cells of different sources of SC, from co-products of alcoholic fermentation, has high efficiency (>90%) to bind AFB_1 in PBS in a relatively short period, as there were no differences in the toxin binding between the contact times of 5, 10, 20 or 30 min.

- Therefore the methods of aflatoxin removal employing SC have a potential application for reducing the levels of AFB_1.

Conclusion

– However, additional studies are needed to investigate the mechanisms involved in the removal process of toxin due SC and the factors that affect the stability of the toxin sequestration aiming the commercial application in the food industry.

Thank you for your attention

Prof. Carlos Humberto Corassin

DVM, Ms, PhD.

Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo - Brazil

carloscorassin@usp.br