

Evaluation of Anti-Aflatoxin M₁ effects of heat-killed cells of *Saccharomyces cerevisiae* in Brazilian commercial yogurts

Rogério C. Pires², Marina R. P. Portinari¹, Giulia Z. Moraes¹, Amin Mousavi Khaneghah³, Bruna L. Gonçalves¹, Roice E. Rosim¹, Carlos A. F. Oliveira¹, Carlos H. Corassin¹

¹Department of Food Engineering, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, Brazil; ²Department of Animal Science Engineering, College of Agriculture Luiz de Queiroz of University of Sao Paulo, Piracicaba, Brazil; ³Department of Food Science, Faculty of Food Engineering, State University of Campinas, Campinas, Brazil

Correspondence Author: Carlos A. F. Oliveira. Av. Duque de Caxias Norte 225, Campus USP, Pirassununga, SP, Brazil, CEP 13635-900. Email: carlosaf@usp.br

Received: 10 November 2021; Accepted: 30 January 2022; Published: 19 February 2022 © 2022 Codon Publications



ORIGINAL ARTICLE

Abstract

This study aimed to assess the aflatoxin M_1 (AFM₁) levels in 72 samples of yogurt from eight processing plants in São Paulo, Brazil, and the ability of heat-killed cells of *Saccharomyces cerevisiae* (10¹⁰ yeast cells/g) to reduce AFM₁ (0.5 µg/kg) in experimental yogurts (n = 3). Analyses were conducted by high performance liquid chromatography (HPLC). Only seven samples (9.8%) had AFM₁ at a mean level of 0.071 \pm 0.08 µg/kg. *S. cerevisiae* efficiently reduced (P < 0.05) the AFM₁ levels in spiked yogurts, with a maximum reduction of 46% after 30 days of storage. Further studies should investigate potential effects of *S. cerevisiae* on the sensory properties of yogurts.

Keywords: AFM,; decontamination; Saccharomyces cerevisiae; yeasts; yogurts

Introduction

Aflatoxins are the most known and vastly distributed mycotoxins in food and feed products, being synthesized by fungi species from the genus Aspergillus, especially A. flavus, A. parasiticus, and A. nomius (Wochner et al., 2018). Although more than 20 types of aflatoxin have been identified, aflatoxin B₁ (AFB₁) is accounted as the main toxic metabolite produced by fungi in naturally contaminated cereals and other food products, as well as in animal feed. AFB, is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (2002). Feeding dairy cows with any ingredient contaminated with AFB, can result in the further conversion of the parent composite into aflatoxin M, (AFM₁), which is excreted in urine and milk (Gonçalves et al., 2015). In milk, AFM, is associated with casein, which persists bound to the toxin during the production of dairy products, including powdered milk, cheese, and yogurt (Campagnollo *et al.*, 2016; Kuharic *et al.*, 2018; Makhdoumi *et al.*, 2021). Besides, AFM₁ in milk or dairy products cannot be completely removed by regular heat treatments, like pasteurization or sterilization (Assaf *et al.*, 2019; Campagnollo *et al.*, 2016; Muaz *et al.*, 2021; Ondiek *et al.*, 2022). However, previous studies indicate that AFM₁ levels in milk can be reduced by the addition of yeast cells of *Saccharomyces cerevisiae*, in view of the ability of this yeast species to absorb and/or inactivate AFM₁ (Corassin *et al.*, 2013).

S. cerevisiae is one of the most important yeasts used in the food industry, also being considered a GRAS ("generally recognized as safe") organism (Van der Hoek *et al.*, 2019). Thus, a biological approach for reducing aflatoxin based on *S. cerevisiae* strains that are already used in food products is an attractive alternative to reduce the AFM₁

levels in yogurt and other fermented dairy products. The incorporation of nonviable cells of *S. cerevisiae* in Minas Frescal cheese, alone or in combination with lactic acid bacteria, resulted in up to 100% reduction of AFM₁ in this type of cheese after 20 days of storage (Gonçalves *et al.*, 2020). Furthermore, some yeast species have probiotic properties, including resistance to the acidified medium of stomach and ability to improve the gut microbiota (Souza *et al.*, 2021). *S. boulardii* and *Pichia kudriavzevii* have been added to beverages (Paula *et al.*, 2019) and cereal-based fermented foods (Greppi *et al.*, 2017), respectively, to provide beneficial effects to the human host, thus opening new perspectives for the development of innovative yeast-based functional food products.

Milk and dairy products are essential segments of the human diet, being largely consumed by people of different age groups, especially the elderly and children (Campagnollo et al., 2016). Therefore, the occurrence of AFM, in milk and milk products represents a notable hazard to human health (Gonçalves et al., 2020; Souza et al., 2020; Sumon et al., 2021). In this context, several studies revealed that human exposure to the aflatoxins may be increased through consumption of AFM,contaminated milk and dairy products (Campagnollo et al., 2016; Gonçalves et al., 2021; Hassan and Kassaify, 2014; Makhdoumi et al., 2021; Womack et al., 2016). In Brazil, some studies regarding the occurrence of AFM, showed high incidence of contaminated samples, ranging from 63 to 100%, and levels ranging from 0.0002 to 0.106 µg/L among different yogurt and other milk products (Gonçalves et al., 2021; Iha et al., 2011; Picinin et al., 2013). Despite these limited occurrence data, there is no information on the frequency and levels of AFM, in yogurt collected directly from Brazilian dairy producers.

Yogurt is obtained by natural fermentation of whole or standardized milk with Lactobacillus delbruecki subsp. bulgaricus and Streptococcus thermophilus (Cruz et al., 2013). In addition, yogurt is one of the most consumed fermented milks in Brazil (Iha et al., 2011), and it is also an excellent vehicle for delivering probiotics (Cruz et al., 2013) and prebiotics (Muaz et al., 2021). Therefore, it can be hypothesized that the addition of yeasts in the manufacture of yogurts may reduce the AFM, levels in the contaminated product. This is in accordance with the need for safe and practical decontamination methods that are acceptable to consumers and can be applied during biotechnological processes of fermented foods such as yogurts (Piotrowska et al., 2021). However, the addition of S. cerevisiae cells into yogurts to decontaminate AFM, in the final product has never been explored. In this context, the present study aimed to determine the occurrence of AFM, in yogurt samples collected from eight different dairy processing plants in São Paulo state, Brazil, and to evaluate the ability of S. cerevisiae to reduce the $\mathsf{AFM}_{\scriptscriptstyle 1}$ levels in spiked yogurt with or without the addition of yeast.

Material and Methods

Assessment of aflatoxin M₁ in yogurt manufactured in dairy processing plants

Sampling procedures were carried out in eight yogurt processing plants located in the northeastern region of the state of São Paulo, Brazil. A total of 72 yogurt samples were collected (n = 9, for each plant). In each factory, nine batches of yogurt production were sampled, totaling 72 batches of yogurt evaluated in the study. All collected samples were transported to the laboratory in a thermal box with dry ice and stored at 4°C until AFM $_{\text{I}}$ determination analysis.

Assessment of the ability of *S. cerevisiae* to reduce aflatoxin M₄ in yogurt

Twelve yogurt samples (1-L bottles) from the same lot and the same manufacturer were purchased from a local supermarket and used to evaluate the ability of S. cerevisiae to reduce AFM, in the product. All yogurt samples were formerly analyzed and considered free of AFM, (below the detection limit of the analytical method: 0.017 μg/kg). Each yogurt sample was assigned to one treatment in a completely randomized study using a factorial arrangement of 2 × 2, corresponding to two levels of S. cerevisiae (0 and 1010 yeast cells/kg yogurt) and two levels of AFM, (0 and 0.5 µg/kg yogurt), totaling four treatments with three repetitions per treatment. The two levels of S. cerevisiae (0 and 1010 yeast cells/kg yogurt) were selected based on previous studies on the application of this yeast for AFM, decontamination in milk (Corassin et al., 2013) and cheese (Gonçalves et al., 2021).

The *S. cerevisiae* strain (categorized as a GRAS organism) used for incorporation into the yogurts was a commercially available brewer's biological dry yeast (Fermentis K-97, SafAle, Bruggeman, Belgium) containing 1.0×10^{10} cells/g. Prior to the addition to yogurts, the cells of *S. cer*evisiae were submitted for inactivation in an autoclave at 121°C for 10 min, to avoid any effect on the fermentation of yogurt. The AFM, used (Sigma-Aldrich, USA) was previously diluted in acetonitrile at 0.5 µg/mL. An aliquot of 0.5 mL of this solution was evaporated in a flask under nitrogen flow, then 0.5 kg of yogurt and 0.5 g of the heatkilled yeast cells biomass were added in the flask and mixed thoroughly for 15 min, to obtain the required levels of AFM, and yeast in the prepared yogurts. The prepared yogurts were stored at 4°C for 30 days, and samples were collected immediately and after preparation (day 0) and at 10-day intervals.

Determination of aflatoxin M, in yogurt

AFM $_1$ was extracted and purified from all yogurt samples (collected in dairy plants and artificially spiked with AFM $_1$ and/or yeast cell biomass) using immunoaffinity columns (Aflatest WB, Vicam, Watertown, MA, USA), exactly as described by Jager *et al.* (2013). Final extracts from yogurt samples were injected (20 μ L) into a Shimadzu 10VP liquid chromatograph (Kyoto, Japan), equipped with a 10 AXL fluorescence detector (excitation at 360 nm and emission above 440 nm). The chromatographic run was achieved using a Kinetex C_{18} column (Phenomenex, Torrance, CA, USA) 4.6 × 150 mm, 2.6 μ m particle size, and the isocratic mobile phase consisted of methanol/water/acetonitrile (61.4:28.1:10.5, v/v/v) with a flow rate of 0.50 mL/min.

Five-point calibration curves containing AFM_1 at levels from 0.1 to 1.0 μ g/L were prepared using AFM_1 standard prepared in acetonitrile. Integrated peak areas were linearly correlated with the concentrations. Identification of AFM_1 was achieved by comparing the retention time of AFM_1 peaks in the samples with the standards in the calibration curves. The limits of detection (LOD) and limits of quantification (LOQ) were calculated at a signal-tonoise ratio of 3 and 10, respectively, being 0.017 and 0.055 μ g/kg, respectively. The analytical method was previously validated with contaminated yogurt samples at levels of 0.2 and 0.5 μ g/kg (n = 3, for each concentration), which resulted in AFM_1 recovery rates in yogurt samples ranging from 72 to 93% (Jager *et al.*, 2013).

Analysis of the pH of spiked yogurts

The pH was determined in yogurt samples artificially spiked with AFM_1 and/or yeast cell biomass as described by AOAC (2019).

Statistical analysis

The General Linear Model of SAS (2004) was approached as the statistical analysis of AFM_1 binding assays, while a level of P < 0.05 was considered as significant.

Results and Discussion

The occurrence of aflatoxin M₁ in yogurt collected in dairy plants

AFM $_1$ was detected in seven samples (9.8 %) of yogurt manufactured in dairy plants at São Paulo state, with a range of 0.017 to 0.130 $\mu g/kg$ (Table 1). While no regulation for the levels of AFM $_1$ in yogurt was established

Table 1 Aflatoxin M₁ (AFM₁) levels in yogurt manufactured in dairy plants at São Paulo, Brazil.

Range of AFM ₁ level (μg/kg)	Number of samples	%
<lod<sup>a</lod<sup>	65	90.2
	00	
LOD-0.05	4	5.6
0.05–0.25	3	4.2
0.25-0.50	0	0
0.50–1.00	0	0
Total	72	100

aLOD: Limit of detection (0.017 µg/kg).

in Brazil, none of the analyzed samples presented levels higher than the Brazilian limit for milk (0.50 µg/L) (ANVISA, 2011). As AFM $_{\rm l}$ is frequent in dairy foods produced worldwide, many countries proposed some regulatory limits for AFM $_{\rm l}$ in milk and dairy products, with limits varying from 0.05 to 0.5 µg/kg (Iha et~al.,~2011). Studies have described the occurrence of AFM $_{\rm l}$ in yogurt worldwide, although the frequency is high; in most studies, the reported levels of AFM $_{\rm l}$ were considered low (Muaz et~al.,~2021; Souza et~al.,~2020).

The number of the contaminated samples (n = 7) and the mean level of AFM, $(0.051 \pm 0.13 \mu g/kg)$ reported in the present study were similar to those reported by Cano-Sancho et al. (2010), who evaluated the occurrence of AFM, in 72 samples of yogurt marketed in Spain and detected a low incidence of AFM₁, 2.8% (n = 2), and low levels of AFM₁, ranging from 0.04 to 0.052 µg/kg. However, in Iran, Fallah (2010) and Nilchian and Rahumi (2012) reported a higher incidence of AFM, in yogurt, about 66.1% (n = 45) and 35% (n = 14), respectively. However, both studies reported ranges for AFM₁ of 0.015 to 0.119 µg/kg, and 0.011 to 0.116 µg/kg, respectively. Analogous to Iran, in Pakistan, Iqbal et al. (2013) reported a higher incidence of AFM₁, 33.3% (n = 32), than in the present study and low levels of AFM, (0.019 to 0.053 µg/ kg) in the evaluated yogurt samples. In Turkey, as well as in Pakistan, Ertas et al. (2011) and Kocasari et al. (2012) reported a high incidence of AFM₁ in the samples, 56% (n = 28) and 44.4% (n = 20), and low levels of AFM₁ 0.002 $\mu g/kg$ at 0.078 and 0.05 to 0.36 $\mu g/kg$, respectively. In Qatar, Hassan et al. (2018), despite reporting a high incidence of 76% (n = 16), the levels of AFM, detected in the yogurt samples were less than $0.05 \mu g/L$.

Several reports indicate that the occurrence of AFM₁ in milk and dairy products strongly depended on several factors, including lactation stage, feed quality, season/climate, animal breed, and milk production performance beside the used technique for AFM₁ assessment (Hassan *et al.*, 2018; Iqbal *et al.*, 2017;

Makhdoumi et al., 2021; Shahbazi et al., 2017). Considering the findings from studies conducted in Spain, Iran, Pakistan, Turkey, Qatar, and Brazil, the incidences of AFM, in yogurt are greater than that noted in the present study (Makhdoumi et al., 2021; Muaz et al., 2021; Souza et al., 2020; Sumon et al., 2021). However, the levels in the aforementioned studies were overall low, similar to our data, thus indicating low exposure to AFM, through intake of these products. Although in our study a limited number of samples was screened, the results indicate that milk received for the manufacture of vogurt in the dairy plants evaluated have low incidence and levels of AFM₁. These findings stress the need for control measures to avoid fungi growth and AFB, formation in dairy farms to prevent milk contamination with AFM, (Gonçalves et al., 2017). Good agricultural practices, which include the use of pest-resistant crops, proper cultivation practices, proper use of fertilizers, irrigation, and crop rotation, are essential tools to prevent and control mycotoxins in dairy farms (Gonçalves et al., 2015).

Evaluation of the ability of *S. cerevisiae* to reduce aflatoxin M, in yogurt

The pH of the yogurt, stored at 4° C, was not affected (P > 0.05) by using *S. cerevisiae* in any of the evaluated treatments, during the entire period (from days 0 to 30) of the study (Table 2).

As expected, AFM₁ concentrations in nonspiked yogurts were below the LOD of the analytical method (0.017 µg/kg). The mean levels of AFM₁ in spiked yogurts ranged from 0.27 \pm 0.03 to 0.50 \pm 0,01 µg AFM₁/kg during 30 days of storage (Table 3). In our study, in the treatment without *S. cerevisiae*, a percentage reduction of 10% in AFM₁ after 30 days was noted, which can be associated with the natural function of lactic acid bacteria in raw and pasteurized milk used in the processing of yogurt (Franciosi *et al.*, 2009). Another explanation for the observed reduction in AFM₁ can be correlated with the low pH value. Corroborating with our study, Govaris *et al.* (2002) reported the stability of AFM₁ in yogurt

Table 2. pH values of yogurts prepared with or without the addition of heat-killed cells of yeast and aflatoxin M, during 30 days of storage.

Yeast ^a (cells/kg)	AFM ₁ (µg/kg)	рН					
		Day 0	Day 10	Day 20	Day 30	Mean ^b	
0	0	4.01	4.14	4.03	4.05	4.06 ± 0.06	
10 ¹⁰	0	4.35	4.34	4.28	4.3	4.32 ± 0.03	
0	0.5	4.03	4.01	3.95	3.99	4.00 ± 0.03	
10 ¹⁰	0.5	4.92	4.34	4.19	4.24	4.42 ± 0.34	

^aCommercially available brewer's biological dry yeast (Fermentis K-97, SafAle, Bruggeman, Belgium) containing 1.0 × 10¹⁰ yeast cells/g.

Table 3. Mean aflatoxin M₁ (AFM₁) levels and percentage reductions (R) in spiked yogurts prepared with or without heat-killed cells of yeast during 30 days of storage.

Yeast ^a (cells/kg)	AFM₁ (µg/kg)	Aflatoxin M ₁ in yogurt during storage							
		Day 0		Day 10		Day 20		Day 30	
		Mean level (μg/kg) ^b	% (R) ^c	Mean level (μg/kg)	% (R)	Mean level (μg/kg)	% (R)	Mean level (μg/kg)	% (R)
0	0	<lod<sup>d</lod<sup>	_	<lod< td=""><td>_</td><td><lod< td=""><td>_</td><td><lod< td=""><td>_</td></lod<></td></lod<></td></lod<>	_	<lod< td=""><td>_</td><td><lod< td=""><td>_</td></lod<></td></lod<>	_	<lod< td=""><td>_</td></lod<>	_
10 ¹⁰	0	<lod< td=""><td>_</td><td><lod< td=""><td>_</td><td><lod< td=""><td>_</td><td><lod< td=""><td>-</td></lod<></td></lod<></td></lod<></td></lod<>	_	<lod< td=""><td>_</td><td><lod< td=""><td>_</td><td><lod< td=""><td>-</td></lod<></td></lod<></td></lod<>	_	<lod< td=""><td>_</td><td><lod< td=""><td>-</td></lod<></td></lod<>	_	<lod< td=""><td>-</td></lod<>	-
0	0.5	0.50 ± 0.01	0.0	0.49 ± 0.03	2.0	0.48 ± 0.02	4.0	0.45 ± 0.01	10.0
1010	0.5	0.46 ± 0.01	8.0	0.38 ± 0.01	24.0	0.32 ± 0.01	36.0	0.27 ± 0.03	46.0

^aCommercially available brewer's biological dry yeast (Fermentis K-97, SafAle, Bruggeman, Belgium) containing 1.0 × 10¹⁰ yeast cells/g.

^bValues were expressed as mean ± standard deviation of samples analyzed in triplicate.

No significant differences were found between means in rows or columns (P > 0.05).

^bValues are expressed as mean ± SD of samples analyzed in triplicate.

[°]Cumulative reduction percentages of AFM, in relation to the initial concentration of AFM, in spiked yogurts.

dLOD: Limit of detection (0.017 µg/kg).

artificially contaminated with concentrations of 0.05 and 0.1 μ g/L, during storage for 4 weeks, at 4°C, at two pH levels (4.0 and 4.6). Their findings demonstrated that at a pH of 4.6, no significant change in AFM₁ levels was observed. However, AFM₁ showed a significant decrease after the third and fourth weeks of storage. The authors quoted that the reduction of AFM₁ could be a function of the low pH.

The effect of S. cerevisiae in reducing AFM, was highlighted by the findings of our study. There was an 8% reduction in AFM, in yogurt on day 0, followed by an increase in reduction on day 10 (24%), continuing the reduction on day 20 (36%), and at day 30, the percentage of AFM, decreased, reaching a reduction of 46%. There is only one previous study that assessed the effect of S. cerevisiae on the removal of aflatoxin M, in yogurt. In a similar study to ours, Karazhiyan et al. (2016) reported AFM, reduction percentages much higher than ours, when they evaluated the ability of S. cerevisiae (viable, treated with acid, heat, and ultrasound) to bind to AFM, in yogurt over time (days 1, 7, 14, and 21 after manufacture). Among the treated yeasts, the one with the highest binding capacity to AFM, was treated with acid (76.46%). Yeasts treated with heat (76.39%) and ultrasound (74.20%) also showed high percentages of reduction. An important advantage of using *S. cerevisiae* as the AFM, binder in yogurts is the overall acceptance of this yeast without restrictions in the food industry, considering its classification as a GRAS organism (Van der Hoek et al., 2019). Besides, the low costs of adding S. cerevisiae biomass in yogurts provide a viable alternative to the dairy industry to reduce the AFM, contamination in the product during the storage period. In this regard, further investigations are recommended to evaluate the involved mechanisms in the process of mycotoxin reduction by S. cerevisiae. In addition, the associated factors with the stability of the sequestration of toxins, such as the concentration of yeasts, acidity, and type of initial culture, should be considered (Karazhiyan et al., 2016).

Conclusion

The limited survey performed in the present study indicates that milk received for the manufacture of yogurt in the dairy plants evaluated have low incidence (9.8%) and levels (mean: $0.071 \pm 0.08 \, \mu g/kg$) of AFM₁. The addition of *S. cerevisiae* biomass in yogurts containing $0.5 \, \mu g/kg$ of AFM₁ reduced its concentration to $0.27 \, \mu g/kg$ after 30 days of storage, thus providing a 46% decrease of AFM₁ in the period. Results of this trial indicate that the incorporation of *S. cerevisiae* could efficiently decrease the AFM₁ levels in yogurt. Further studies are required to examine the involved mechanisms in the process of aflatoxin reduction by *S. cerevisiae*. In addition, the associated

factors with the stability of the sequestration of toxins, such as the concentration of yeasts, acidity, and type of initial culture, should be considered.

Acknowledgements

This research was funded by São Paulo Research of Foundation, FAPESP (grant number 2017/20081-6), by the Brazilian National Council for Scientific and Technological Development, CNPq (Grant # 302195/2018-1), and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001.

References

- AOAC Association of Official Analytical Chemists, 2019. Official methods of analysis. 19th ed. Association of Official Analytical Chemists, Washington, DC.
- Assaf, J., Nahle, S., Chokr, A., Louka, N., Atoui, A. and El Khoury, A., 2019. Assorted methods for decontamination of aflatoxin M_1 in milk using microbial adsorbents. Toxins 11: 1–23. https://doi.org/10.3390/toxins11060304
- ANVISA Agência Nacional de Vigilância Sanitária, 2011. Resolução RDC nº 7, de 18 de fevereiro de 2011. Diário Oficial da União Seção 1 37: 72–73. Available at: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2011/res0007_18_02_2011_rep.html.
- Campagnollo, F., Ganev, K., Khaneghah, A., Portela, J., Cruz, A., Granato, D., Corassin, C., Oliveira, C. and Sant'Ana, A.S., 2016. The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M_1 : a review. Food Control 68: 310–329. https://doi.org/10.1016/j.foodcont.2016.04.007
- Cano-Sancho, G., Marin, S., Ramos, A., Peris-Vicente, J. and Sanchis, V., 2010. Occurrence of aflatoxin M₁ and exposure assessment in Catalonia (Spain). Ibero-American Journal of Mycology 27: 130–135. https://doi.org/10.1016/j.riam.2010.05.003
- Corassin, C., Bovo, F., Rosim, R. and Oliveira, C., 2013. Efficiency of *Saccharomyces cerevisiae* and lactic acid bacteria strains to bind aflatoxin M₁ in UHT skim milk. Food Control 31: 80–83. https://doi.org/10.1016/j.foodcont.2012.09.033
- Cruz, A., Cavalcanti, R., Gherreiro, L., Sant'ana, A., Nogueira, L., Oliveira, C., Deliza, R., Cunha, R., Faria, J. and Bolini, H., 2013. Developing a prebiotic yogurt: rheological, physico-chemical and microbiological aspects and adequacy of survival analysis methodology. Journal of Food Engineering 114: 323–330. https://doi.org/10.1016/j.jfoodeng.2012.08.018
- Ertas, N., Gonulalan, Z., Yildirim, Y. and Karadal, F., 2011. A survey of concentration of aflatoxin $\rm M_1$ in dairy products marketed in Turkey. Food Control 22: 1956–1959. https://doi.org/10.1016/j. foodcont.2011.05.009
- Fallah, A., 2010. Aflatoxin M₁ contamination in dairy products marketed in Iran during winter and summer. Food Control 21: 1478–1481. https://doi.org/10.1016/j.foodcont.2010.04.017
- Franciosi, E., Settanni, L., Cavazza, A. and Poznanski, E., 2009. Biodiversity and technological potential of wild lactic acid

- bacteria from raw cows' milk. International Dairy Journal 19: 3–11. https://doi.org/10.1016/j.idairyj.2008.07.008
- Gonçalves, B., Corassin, C. and Oliveira, C., 2015. Mycotoxicoses in dairy cattle: a review. Asian Journal of Animal and Veterinary Advance 10: 752–760. https://doi.org/10.3923/ajava.2015.752.760
- Gonçalves, B., Gonçalves, J., Rosim, R., Cappato, L., Cruz, A., Oliveira, C. And Corassin, C., 2017. Effects of different sources of *Saccharomyces cerevisiae* biomass on milk production, composition, and aflatoxin M₁ excretion in milk from dairy cows fed aflatoxin B₁. Journal of Dairy Science 100: 5701–5708. https://doi.org/10.3168/jds.2016-12215
- Gonçalves, B., Muaz, K., Coppa, C., Rosim, R., Kamimura, E., Oliveira, C. and Corassin, C., 2020. Aflatoxin M₁ absorption by non-viable cells of lactic acid bacteria and *Saccharomyces cerevisiae* strains in Minas Frescal cheese. Food Research International 136: 109604. https://doi.org/10.1016/j.foodres.2020.109604
- Gonçalves, B., Uliana, R., Coppa, C., Lee, S., Kamimura, E., Oliveira, C. and Corassin, C., 2021. Aflatoxin M_1 : biological decontamination methods in milk and cheese. Food Science and Technology 4: 1–8. https://doi.org/10.1590/fst.22920
- Govaris, A., Roussi, V., Koidis, P. and Botsoglou, N., 2002. Distribution and stability of aflatoxin M_1 during production and storage of yoghurt. Food Additives and Contaminants 19: 1043–1050. https://doi.org/10.1080/0265203021000007831
- Greppi, A., Saubade, F., Botta, C., Humblot, C., Guyot, J.P. and Cocolin, L., 2017. Potential probiotic Pichia kudriavzevii strains and their ability to enhance folate content of traditional cerealbased African fermented food. Food Microbiology 62: 169–177. https://doi.org/10.1016/j.fm.2016.09.016
- Hassan, H. and Kassaify, Z., 2014. The risks associated with aflatoxin M₁ occurrence in Lebanese dairy products. Food Control 37: 68–72. https://doi.org/10.1016/j.foodcont.2013.08.022
- Hassan, Z., Al-Thani, R., Atia, F., Almeer, S., Balmas, V., Migheli, Q. and Jaoua, S., 2018. Evidence of low levels of aflatoxin M_1 in milk and dairy products marketed in Qatar. Food Control 92: 25–29. https://doi.org/10.1016/j.foodcont.2018.04.038
- Iha, M., Barbosa, C., Okada, I. and Trucksess, M., 2011. Occurrence of aflatoxin $\rm M_1$ in dairy products in Brazil. Food Control 22: 1971–1974. https://doi.org/10.1016/j.foodcont.2011.05.013
- International Agency for Research on Cancer, 2002. IARC Monographs of the evaluation of carcinogenic risks to humans, vol. 82: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC, Lyon, France, pp. 171-300.
- Iqbal, S., Asi, M. and Jinap, S., 2013. Variation of aflatoxin M_1 contamination in milk and milk products collected during winter and summer seasons. Food Control 34: 714–718. https://doi.org/10.1016/j.foodcont.2013.06.009
- Iqbal, S., Asi, M. and Malik, N., 2017. The seasonal variation of aflatoxin $\rm M_1$ in milk and dairy products and assessment of dietary intake in Punjab, Pakistan. Food Control 79: 292–296. https://doi.org/10.1016/j.foodcont.2017.04.015
- Jager, A., Tedesco, M., Souto, P. and Oliveira, C., 2013. Assessment of aflatoxin intake in São Paulo, Brazil. Food Control 33: 87–92. https://doi.org/10.1016/j.foodcont.2013.02.016

- Karazhiyan, H., Mehraban Sangatash, M., Karazhyan, R., Mehrzad, A. and Haghighi, E., 2016. Ability of different treatments of Saccharomyces cerevisiae to surface bind aflatoxin M_1 in yoghurt. Journal of Agricultural Science and Technology 18: 1489–1498.
- Kocasari, F., Tasci, F. and Mor, F., 2012. Survey of aflatoxin M₁ in milk and dairy products consumed in Burdur, Turkey. International Journal Dairy Technology 65: 365–371. https://doi.org/10.1111/j.1471-0307.2012.00841.x
- Kuharic, Z., Jakopovic, Z., Canak, I., Frece, J., Bosnir, J., Pavlek, Z., Ivesic, M. and Markov, K., 2018. Removing aflatoxin M₁ from milk with native lactic acid bacteria, centrifugation, and filtration. Archives of Industrial Hygiene and Toxicology 69: 334–339. https://doi.org/10.2478/aiht-2018-69-3160
- Makhdoumi, P., Hossini, H., Mohammadi, R. and Limoee, M., 2021. The prevalence of aflatoxin M_1 (AFM $_1$) in conventional and industrial dairy products (yogurt, cheese, kashk and dough) of Iran: a systematic review and meta-analysis. Reviews on Environmental Health 28: 1–11. https://doi.org/10.1515/reveh-2021-0028
- Muaz, K., Riaz, M., Rosim, R., Akhtar, S., Corassin, C., Gonçalves, B. and Oliveira, C., 2021. In vitro ability of nonviable cells of lactic acid bacteria strains in combination with sorbitan monostearate to bind to aflatoxin M₁ in skimmed milk. LWT-Food Science and Technology 147: 111666. https://doi.org/10.1016/j. lwt.2021.111666
- Nilchian, Z. and Rahumi, A., 2012. Aflatoxin $\rm M_1$ in yoghurts, cheese and ice-cream in Shahrekord-Iran. World Applied Science Journal 19: 621–624.
- Ondiek, W., Wang, Y., Sun, L., Zhou, L., On, S.L., Zheng, H. and Ravi, G., 2022. Removal of aflatoxin B₁ and T-2 toxin by bacteria isolated from commercially available probiotic dairy foods. Food Science and Technology International 28: 15–25. https://doi.org/10.1177/1082013220987916
- Paula, B.P., Chávez, D.W.H., Junior, W.J.F.L., Guerra, A.F., Corrêa, M.F.D., Pereira, K.S. and Coelho, M.A.Z., 2019. Growth parameters and survivability of *Saccharomyces boulardii* for probiotic alcoholic beverages development. Frontiers in Microbiology 10: 1–10. https://doi.org/10.3389/fmicb.2019.02092
- Picinin, L., Cerqueira, M., Vargas, E., Lana, Â., Toaldo, I. and Bordignon-Luiz, M., 2013. Influence of climate conditions on aflatoxin M₁ contamination in raw milk from Minas Gerais State, Brazil. Food Control 31: 419–424. https://doi.org/10.1016/j. foodcont.2012.10.024
- Piotrowska, M., 2021. Microbiological decontamination of mycotoxins: opportunities and limitations. Toxins 13: 1–19. https://doi.org/10.3390/toxins13110819
- SAS Institute, 2004. SAS User's guide: statistics. SAS Institute Inc., Cary, NC.
- Shahbazi, Y., Nikousefat, Z. and Karami, N., 2017. Occurrence, seasonal variation and risk assessment of exposure to aflatoxin M_1 in Iranian traditional cheeses. Food Control 79: 356–362. https://doi.org/10.1016/j.foodcont.2017.04.021
- Souza, C., Khaneghahi, A. and Oliveira, C., 2020. The occurrence of aflatoxin M, in industrial and traditional fermented milk:

- a systematic review study. Italian Journal of Food Science 33: 53–64. https://doi.org/10.15586/ijfs.v33iSP1.1982
- Souza, H.F., Carosia, M.J., Pinheiro, C., Carvalho, M.V., Oliveira, C.A.F and Kamimura, E.S., 2021. On probiotic yeasts in food development: *Saccharomyces boulardii*, a trend. Food Science and Technology Ahead of print: 1–7. https://doi. org/10.1590/fst.92321
- Sumon, A., Islam, F., Mohanto, N., Kathak, R., Molla, N., Rana, S., Degen, G. and Ali, N., 2021. The presence of aflatoxin $\rm M_1$ in milk and milk products in Bangladesh. Toxins 13: 440–445. https://doi.org/10.3390/toxins13070440
- Van der Hoek, S., Darbani, B., Zugaj, K., Prabhala, B., Biron, M., Randelovic, M., Medina, J., Kell, D. and Borodina, I., 2019.

- Engineering the yeast *Saccharomyces cerevisiae* for the production of L-(+)-ergothioneine. Frontiers in Bioengineering and Biotechnology 11: 262. https://doi.org/10.1101/667592
- Wochner, K., Becker-Algeri, T., Colla, E., Badiale-Furlong, E. and Drunkler, D., 2018. The action of probiotic microorganisms on chemical contaminants in milk. Critical Review Microbiology 44: 112–123. https://doi.org/10.1080/1040841X.2017.1329275
- Womack, E., Sparks, D. and Brown, A., 2016. Aflatoxin $\rm M_1$ in milk and milk products: a short review. World Mycotoxin Journal 9: 305–315. https://doi.org/10.3920/WMJ2014.1867