

EVALUATION OF OCHRATOXIN A DETOXIFICATION IN GRAPE JUICE BY *Lacticaseibacillus casei* AND *Lactobacillus acidophilus*

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RESUMO – Ocratoxina A (OTA) é um metabólito secundário produzido por fungos toxigênicos com frequente incidência em produtos à base de uva, como o suco de uva, sendo responsável por desencadear diversos efeitos adversos. Na tentativa de reverter estes danos, a redução dessa micotoxina por probióticos derivados de LABs é uma alternativa de baixo custo e segura. Nesse estudo, a capacidade de detoxificação de OTA por *Lacticaseibacillus casei* (LC-01) e *Lactobacillus acidophilus* (LA-05) foi avaliada em suco de uva. Após 24 h, foi possível observar que LC-01 e LA-05 (8 log CFU/mL) foram capazes de detoxificar OTA (10 ng/mL) em 18.1% e 16.8%, respectivamente. A detoxificação da micotoxina quando o sistema foi mantido em agitação (100 rpm) foi de 52.4% e 40.0% utilizando LC-01 e LA-05 respectivamente. Portanto, a identificação desses probióticos como agentes de remoção dessa micotoxina abre perspectivas de emprego na indústria alimentícia vinícola.

PALAVRAS-CHAVE: Ocratoxina A, probióticos, detoxificação.

ABSTRACT – Ochratoxin A (OTA) is a secondary metabolite produced by fungi that can contaminate grape-based products, such as grape juice, and is responsible for triggering various diseases. In an attempt to reduce this mycotoxin, probiotics derived from LABs are emerging as an inexpensive and safe alternative. In this study, the detoxification capacity of *Lacticaseibacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05) was tested in grape juice. After 24 hours, it was observed that LC-01 and LA-05 (8 log CFU/mL) were able to detoxicate OTA (10 ng/mL) by 18.1% and 16.8%, respectively. The detoxification of mycotoxin when the system was kept under agitation



(100 rpm) was 52.4% and 40.0% using LC-01 and LA-05, respectively. Therefore, the identification of these probiotics as mycotoxin removal agents opens up prospects for their application in the wine food industry.

KEYWORDS: Ochratoxin A, probiotics, detoxification.

1. INTRODUCTION

Ochratoxin A (OTA), a secondary metabolite produced by species of *Aspergillus* and *Penicillium*, is classified as 2B by the International Agency for Research on Cancer (IARC) (Khan *et al.*, 2024). Grapes, grape juice, wine, and grape-based products are the most common foods contaminated by this mycotoxin (Nora *et al.*, 2019). The maximum allowable concentration of OTA in grape juice is 2 µg/L, as established by European Union and Brazilian legislation (EU, 2006; ANVISA, 2011).

Lactic acid bacteria (LAB) stand out as a potential agent for OTA removal. These microorganisms can remove OTA through adsorption, due to its binding to the cell wall structure, and biodegradation, using enzymes secreted by the microorganism (Adegoke *et al.*, 2022). Defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2002), most of probiotics are derived from LAB.

Studies have shown that probiotics have effects on certain food contaminants, as mycotoxins biodegradation (Khani *et al.*, 2024). Furthermore, the *Lactobacillus* genera are the most common in those removals (Manoj *et al.*, 2023).

This study aimed to evaluate the potential of probiotics obtained from *Lactocaseibacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05) for OTA detoxification in grape juice.

2. MATERIALS AND METHODS

2.1 Grape juice contamination and addition of strains

LC-01 and LA-05, after incubation in MRS broth and anaerobioses, were centrifuged (3500 rpm/15 min/4°C) and washed twice with sterile saline solution. Then, the strains were resuspended in saline solution to the obtention of standard cell suspensions (OD₆₆₀ of 1.0, 8 log CFU/mL). A tube was spiked with OTA 10 ng/mL, added as standard solution of mycotoxin and dried. Then, 1 mL of juice was added to the tube and stored for 24 h at 4°C. The strains were added to the juice until the



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concentration of 8 log CFU/mL. Two treatments were evaluated, with (100 rpm) and without orbital agitation, compared to the control, assay without addition of probiotic. The system was kept in an orbital shaker for 24 h at 25°C.

2.2 OTA extraction and detection

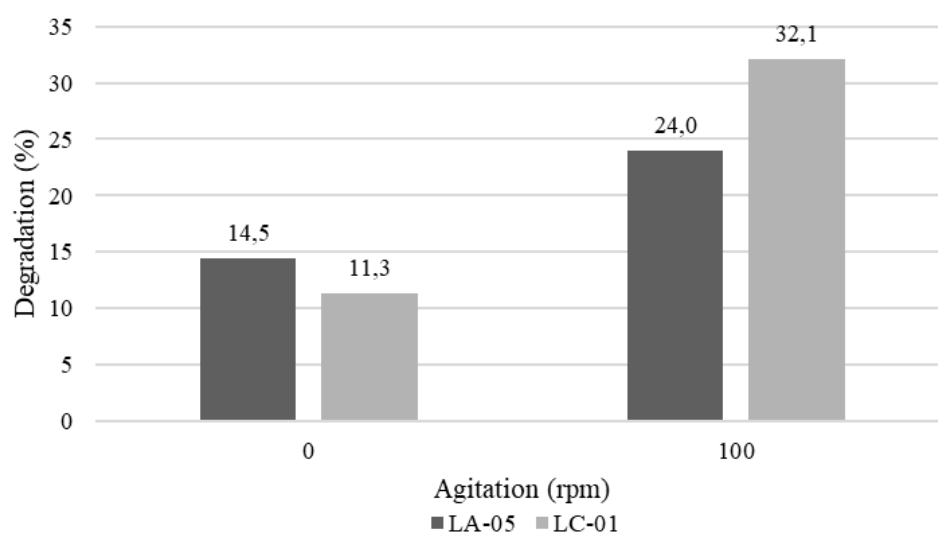
OTA extraction was performed by liquid-liquid partitioning (LLP). Samples were centrifuged, and the supernatant was collected. Afterward, 1.5 mL of chloroform was added to the sample. The mixture was vortexed for 30 s and then sonicated for 3 min in a 25-kHz ultrasonic bath. The organic phase obtained was removed, and the extraction was repeated three times. The final organic phase volume was dried and resuspended in the mobile phase for quantification by HPLC coupled with a fluorescence detector.

3. RESULTS AND DISCUSSION

Grape juice is one of the most consumed grape-based products worldwide. With numerous health benefits, the consumption of this product has increased (Junges et al., 2022). Despite its low levels, the occurrence of OTA poses a risk to humans and animals due to its tendency to accumulate and its carcinogenic potential (Yang et al., 2022).

In this study, OTA detoxification was evaluated using *Lactocaseibacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05). OTA detoxification in grape juice by LC-01 after 24 hours increased when agitation was applied. Meanwhile, when using LA-05 for detoxification, a low OTA removal was observed in non-agitated samples, which increased with agitation after 24 h (Figure 1).

Figure 1 – Detoxification of OTA in grape juice by *Lactocaseibacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05) in 24 h with and without agitation.





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The reduction of OTA in grape juice by probiotics has been studied by Zheng et al. (2023) using *Lactobacillus rhamnosus* Bm01. Reduction of 83.87% when 50 ng/mL was the initial concentration and 100% when 20 ng/mL of OTA was observed after 48 h. The OTA biodegradation capacity of *Lactobacillus acidophilus* was studied by Böhm et al. (2000), and 46% degradation was observed in MRS broth after 48 h. *Lactobacillus acidophilus* and *Lactobacillus casei* were evaluated by Fuchs et al. (2007) in a liquid medium, showing a reduction of 97% and 31%, respectively, at 37°C for 4 h and 500 ng/mL of OTA. These researchers described that the adsorption depends on bacterial concentration and density, highlighted that a more acidic pH leads to higher removal.

The effectiveness of probiotics in OTA detoxification depends on several factors. The binding properties involved in removal are intrinsically related to strain specificity (Nasrollahzadeh et al., 2022). Thus, this study demonstrates that LA-05 and LC-01 have potential as agents for OTA detoxification in grape juice, showing differences between the strains, a considerable removal in a more acidic matrix, and higher reduction under agitation.

As a process primarily based on surface attachment, agitation is responsible for better distribution of the mycotoxin in the medium, increasing solubility and binding with the probiotic (Di Gregorio et al., 2014). Therefore, further analysis is needed to enhance detoxification and define the favorable conditions for the application of these probiotics in this product, as well as to evaluate the probiotic-mycotoxin bond formed through detoxification.

4. CONCLUSION

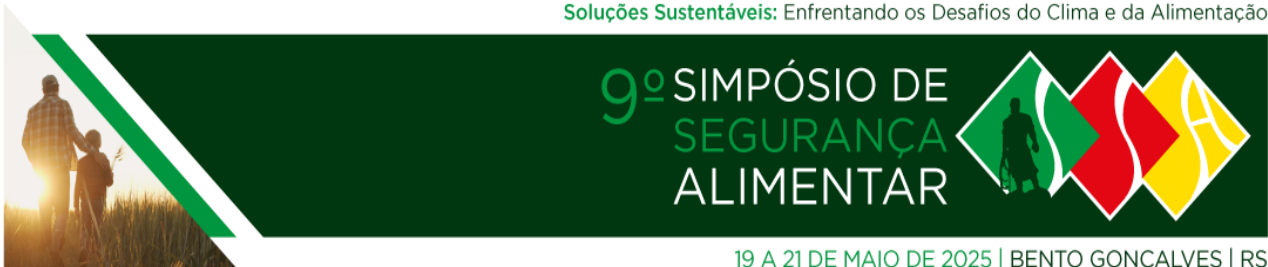
The application of probiotics in foods is already well known for their health benefits. Due to its numerous benefits to human health, the consumption of grape juice has increased in recent years. The combination of these two results in a product that is well accepted by consumers. The use of biological methods to reduce contaminants in these products has increased, particularly against mycotoxins, which are toxic metabolites produced by fungi. In this study, it was possible to observe that probiotics such as *Lactobacillus casei* (LC-01) and *Lactobacillus acidophilus* (LA-05) are capable of detoxifying OTA in grape juice, reducing the amount of this mycotoxin available in the matrix. These results show that these strains can be used as a new approach for OTA detoxification, offering a safe, environmentally friendly, and economical solution.



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