Ochratoxin A reduction by peroxidase in a model system and grape juice

Náthali Saião Nora¹ · Ana Carla Penteado Feltrin¹ · Karen Vanessa Marimón Sibaja¹ · Eliana Badiale Furlong¹ · Jaqueline Garda-Buffon¹

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Abstract

This study aimed at evaluating the potential of the peroxidase (PO) enzyme to reduce ochratoxin A (OTA) levels and its application to grape juice. Both commercial PO and PO extracted from rice bran were evaluated, respectively, regarding their activity towards OTA in a model system. The affinity between PO and OTA was verified by the Michaelis–Menton constant and the maximum velocity parameters, resulting in 0.27 μM and 0.015 $\mu\text{M min}^{-1}$ for the commercial enzyme, and 6.5 μM and 0.031 $\mu\text{M min}^{-1}$ for PO extracted from rice bran, respectively. The lowest residual OTA levels occurred when 0.063 U mL^{-1} of the enzyme was applied. Under these conditions, the OTA reduction was 41% in 5 h for the commercial enzyme, and 59% in 24 h, for PO extracted from rice bran. When the extracted PO, with the activity of 0.063 U mL^{-1} , was applied to whole grape juice, the OTA levels decreased to 17%, at 24 h. The capacity shown by PO for reducing OTA levels was confirmed in whole white grape juice, as a model system. This study may assist the wine industry to offer healthier products and add value to rice bran.

Keywords Enzyme · Mycotoxin degradation · Grape juice

Introduction

Ochratoxin A (OTA) is a compound produced by the secondary metabolism of fungal genera *Aspergillus* and *Penicillium* when the microorganisms go through adverse conditions related to storage and preparation of food products [1, 2]. This

mycotoxin is commonly found in grains, fruits, and their derivatives, such as bakery products, beer, wine, and juices [3–9]. Grape juice, whose intake is strongly recommended as a source of functional compounds, stands out as being prone to mycotoxin contamination. The International Agency for Research on Cancer classified OTA as a possible carcinogen for humans (group 2B) in 1993. The effects include specific nephrotoxicity and others related to hepatotoxicity, teratogenicity, and neurotoxicity. At the molecular level, OTA inhibits protein biosynthesis by competing with phenylalanine in the use of protein positions. OTA has also been related to fertility inhibition and Balkan endemic nephropathy, a chronic kidney disease found in south-eastern Europe [10–12]. The presence of this mycotoxin in food products and the damage it causes to human and animal health have triggered the search for procedures that may decrease its levels and toxic effects. Several decontamination strategies, such as the use of physical and chemical methods, have been proposed [13–15]. However, the extreme conditions used for these methods can affect nutritional values, palatability, and technological properties of raw materials or products [16]. Conversely, biological processes that involve the application of microorganisms or their enzymes have drawn attention

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✉ Jaqueline Garda-Buffon
jaquelinebuffon@furg.br

Náthali Saião Nora
nathali.snora@gmail.com

Ana Carla Penteado Feltrin
aninha.fel@hotmail.com

Karen Vanessa Marimón Sibaja
karenmarimon@hotmail.com

Eliana Badiale Furlong
bafu@vetorial.net

¹ Escola de Química e Alimentos, Laboratório de Micotoxinas e Ciência de Alimentos, Universidade Federal de Rio Grande, Rio Grande, RS, Brazil

because they mitigate the effect of the mycotoxins. These agents promote both toxin degradation and yield metabolites with less toxicity [17–21].

Some microorganisms, such as *Aspergillus oryzae* and *Rhizopus oryzae*, and enzymes, like carboxypeptidase A and lipases, have been investigated for their efficacy as biological mycotoxin-degrading agents [22–25]. However, although researchers have studied the ability of peroxidase (PO) to reduce the toxicity of mycotoxins as zearalenone and deoxynivalenol [1–4], this enzyme has not yet been reviewed regarding its capability to degrade OTA. The PO enzyme can oxidise both organic and inorganic compounds (as electron donors) in the presence of a cofactor, hydrogen peroxide (H_2O_2) [26]. The main source of commercial PO extraction is horseradish (*Armoracia rusticana*) [5]. However, another positive aspect of employing PO is that it may be extracted from alternative sources, such as agro-industrial products. Rice bran (RB), by-products of rice processing, is considered a source of the PO enzyme [1, 3, 6]. As such, it improves the cost-effectiveness of the process and favours large-scale application. Therefore, the potential of the PO enzyme to degrade OTA, besides its application to grape juice, was studied.

Material and methods

Material

The standards, OTA and PO (99% purity), were purchased from Sigma Chemical Company (St. Louis, MO, USA). The stock solution was prepared by dissolving the OTA in benzene:acetonitrile (98:2, v v^{-1}), followed by dilution in benzene:acetic acid (99:1, v v^{-1}), to obtain appropriate working solutions. The solution concentration was determined by the $w \text{ v}^{-1}$ relation and the method described by Kupski and Badiale-Furlon [24], with the use of molar absorptivity ($5550 \text{ L mol}^{-1} \text{ cm}^{-1}$) of the standard at 333 nm. All material was stored at -10°C . Commercial PO was resuspended in Milli-Q water (Millipore, Bedford, MA, USA), diluted, and stored at -20°C . Rice bran, which was used as a source of PO, and the grape juice were purchased at a local market in Rio Grande, RS, Brazil.

Enzyme extraction and purification

The PO enzyme was extracted from rice bran in agreement with a procedure not yet published, by using 5 g of raw material and 50 mL of 10 mM phosphate buffer, pH 5.0. The mixture was shaken at 100 rpm at room temperature for 60 min, centrifuged at $3220\times g$, 4°C for 10 min, filtered, and completed to 50 mL, to obtain the crude enzyme extract. It was purified by the three-phase partitioning (TPP) method described by Özer et al. [27], with modifications. Briefly,

20 mL of the crude enzyme extract was placed in a 50-mL centrifuge tube, saturated with 50% (v v^{-1}) ammonium sulphate at room temperature and then manually homogenised. Acetone was added in the ratio 1:1 (enzyme extract:acetone, v v^{-1}); the mixture was manually homogenised for 1 min and then kept for 1 h for phase separation. Next, the mixture was centrifuged at $3220\times g$ at 4°C for 10 min to facilitate phase separation. Both the top layer (rich in acetone) and the bottom saline portion were removed. Twenty millilitres of 5 mM phosphate buffer, pH 5.5, was added to the precipitated interface containing the enzyme. The solution was manually homogenised for 2 min, filtered, and stored in a freezer at -10°C .

The recovery parameters (REC) and purification factor (PF) were calculated by the following equations, respectively:

$$\text{REC (\%)} = \frac{A_{tEP}}{A_{tEB}} \times 100, \quad (1)$$

where REC is the enzymatic recovery, A_{tEP} is the total activity of the purified extract, and A_{tEB} is the total activity of the crude extract, and

$$\text{PF} = \frac{A_{eEP}}{A_{eEB}}, \quad (2)$$

where PF is the enzymatic purification factor, A_{eEP} is the specific activity (U mg^{-1}) of the purified extract, and A_{eEB} is the specific activity (U mg^{-1}) of the crude extract.

Determination of enzyme activity

The activity of the enzymatic extract, whose mixture of solutions consisted of phosphate buffer, guaiacol, H_2O_2 and water, and the initial reaction when the enzymatic extract was added, were determined as detailed by Devaiah and Shetty [28]. One unit (U) of enzymatic activity was defined as the amount of enzyme that catalyses the reaction of 1 μmol substrate to form 1 μmol of product in 1 min. The specific activity of PO was determined on a protein concentration basis (mg mL^{-1}). The enzyme activity (U mL^{-1}), was calculated, using the following equation [29]:

$$\frac{\text{U}}{\text{mL}} = \frac{\text{Abs} \times f_{dil} \times V_{rxn} \times 10^3}{\varepsilon \times t \times V} \quad (3)$$

where Abs is absorbance, f_{dil} is the dilution sample factor, V_{rxn} (mL) is the reaction volume, 10^3 is the conversion factor from mL to L, ε is the substrate coefficient of molar absorptivity ($26,600 \text{ M}^{-1} \text{ cm}^{-1}$), t is the reaction time (min), and V is the enzymatic volume added in the reaction environment (mL).

Protein concentration was determined by Lowry et al. [30], with absorbance measured at 660 nm. Bovine serum albumin (0–1 mg mL⁻¹) was used to establish the standard curve.

Extraction and quantification of ocratoxin A

For the extraction of OTA, the technique of extraction by liquid-liquid partitioning (LLP) was carried out according to Bauer et al. [7] with some modifications. For this, OTA standard was added and the solvent (benzene: acetonitrile) was evaporated in a nitrogen atmosphere, and the components of model solution (phosphate buffer, water, 0.08% hydrogen peroxide) was added. Then, 3 mL of chloroform (CH₃Cl) was added and vortex agitated for 30 s, 3 min in a 25-kHz ultrasonic bath and centrifugation at 621.6×g for 5 min. The procedure was repeated three times and the organic phase was removed. The final volume of chloroform fraction was evaporated from the samples and resuspended in acetonitrile for detection and quantification.

Chromatographic conditions for OTA detection and quantification

Detection and quantification of the OTA mycotoxin were carried out by high-performance liquid chromatographer (HPLC), manufactured by Shimadzu (Kyoto, Japan), coupled with a fluorescence detector (model FL—10AXL). Detection conditions were according to Kupski; Badiale-Furlong [8]. The chromatographic column was Kromasil (C18.5 µm, 150 mm, 4.6 mm). Solvents that constituted the mobile phase were acetonitrile:acidified water and acetic acid 1% (1:1, v v⁻¹), in isocratic elution. The other parameters used in the method were mobile phase flow of 1 mL min⁻¹ and a column temperature of 35 °C and wavelengths were 333 nm for excitation and 460 nm for emission.

Reductions of the OTA levels

To reduce OTA levels, 0.5 and 0.063 U mL⁻¹ of the commercial PO and PO extracted from rice bran, respectively, were added to the reactor in the presence of 10 ng mL⁻¹ OTA. The standard solution of mycotoxin was added to the reactor before the other constituents (12 h) and the solvent was evaporated under nitrogen flow at room temperature.

The reactor constituents included 16.7 mL of 0.08% H₂O₂ and 33.3 mL of 5 mM phosphate buffer, pH 6.0 (for the commercial enzyme) and pH 5.5 (for PO extracted from rice bran), optimal conditions determined by Feltrin et al. [31] The 100-mL-capacity reactor was completed with water (control), the solution of the purified enzyme extract, and the commercial enzyme diluted in Milli-Q water, respectively. The reactors were kept under orbital shaking at 150 rpm at 25 °C. The residual concentration of the mycotoxin was quantified by collecting aliquots at 0, 15,

30, 60, 300, and 1440 min. The reaction rate was computed, based on Eq. 4, where the reduced OTA (%) was defined as the difference between the initial OTA concentration and those measured at the pre-determined times, respectively.

$$\text{Reaction rate} = \frac{\text{Reduced OTA (ng mL}^{-1}\text{)}}{\text{Reaction time (min)}}. \quad (4)$$

Reduction mechanisms

Physical interaction between protein and OTA was verified after denaturation and hydrolysis by proteases of commercial PO, according to the method described by Angelis et al. [32] with modification. The mycotoxin, the constituents of the reaction system, and the inactive enzyme (80 °C for 60 min) were placed in an Erlenmeyer reactor and orbitally agitated at 150 rpm, at 25 ± 5 °C. To quantify the mycotoxin, aliquots were collected at the initial and final time of catalysis. In the final time, to evaluate the possibility of physical interaction, the enzyme was precipitated by addition of up to 10% trichloroacetic acid, and the mixture was centrifuged at 3220×g at 4 °C for 15 min. The precipitate (inactive enzyme) was suspended in a solution with pepsin (1.5 mg mL⁻¹ in 0.1 M of HCl), and the system was kept at 150 rpm at 37 °C. OTA was quantified at 60, 120, and 180 min, for the inactive enzyme action and after its hydrolysis with pepsin.

$$\frac{1}{v} = \left(\frac{K_M}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}. \quad (5)$$

Enzyme kinetics

Both the Michaelis–Menton constant (K_M) and the maximum velocity (V_{max}) parameters were determined by measuring enzyme action, expressed as the mean velocity of OTA reduction as a function of various mycotoxin concentrations under the reaction conditions used in the reduction of the OTA levels. The OTA concentrations investigated in this experiment were 1.7, 8.3, 15, 18.3, 27.5, and 31.7 ng mL⁻¹ (for the commercial enzyme) and 1.7, 8.3, 15, 18.3, 27.5, and 31.7 ng mL⁻¹ (for PO extracted from rice bran), respectively. The effect of OTA concentration on enzyme activity was described by the K_M , using the Lineweaver–Burk linearization (double-reciprocal plot), where the calculation of K_M and V_{max} values was based on the angular and linear coefficients in Eq. 5, respectively.

Applicability to grape juice

Kinetics of OTA reduction by the action of both commercial PO and PO extracted from rice bran, respectively, was assessed in two types of whole grape juice—red and white—which were

collected at the local market. Determination of juice pH was done in agreement with the Association of Analytical Chemists [33], standard method. At this pH, the enzyme activities were measured. Afterwards, the mycotoxins were extracted and quantified. The analytical performance was evaluated through OTA spiking-recovery, linearity, the correlation and determination coefficients, and the detection and quantification limits.

OTA spiking-recovery was determined by adding 0.7, 3.4, and 20.7 ng mL⁻¹ to white juice, and 3.4, 11.1 and 20.7 ng mL⁻¹ to red juice, respectively. Residual OTA was measured by the method validated for grape juice with matrix recovery assay, linearity, correlation and determination coefficients, and detection limit and the quantification limits of the instrument and method. The enzyme activity was undertaken in an Erlenmeyer reactor with a volume capacity of 100 mL juice. The mycotoxin was added to the reactor, and the solvent was evaporated at room temperature for 12 h. Then, juice and the enzyme cofactor (0.08% H₂O₂) were added at the ratio of 2:1 (w w⁻¹) peroxide:substrate (OTA). The mixture was agitated by ultrasonic waves for 10 min. Enzymes were added, respectively, and orbital agitation maintained at 150 rpm at 25 °C. Sampling was done at 0, 15, 30, 60, 300, and 1440 min.

Statistical analysis

Assays were conducted in triplicate and variations of every experiment were analysed by analysis of variance (ANOVA) and Tukey's test. A value of $p < 0.05$ was considered significant, with the use of the Statistica program (version 5.0, StatSoft, Inc., Tulsa, USA).

Results

Reduction of OTA levels

The reduction of OTA concentration in the model solution (phosphate buffer and H₂O₂) was performed using the commercial PO and PO extracted from rice bran, respectively. The PO derived from rice bran and purified by the TPP technique presented 77.1% for REC and 9.2 for PF. A significant reduction of OTA recovery (Table 1), considering the addition of approximately 0.5 U mL⁻¹ of enzymes, was observed at 1440 min of action for both enzymes. A corresponding 6.0 and 23.5% reduction was obtained for commercial PO and PO extracted from rice bran, respectively.

OTA concentration reduction mechanism

During the hydrolysis, at 60, 120, and 180 min, the concentrations of the mycotoxin remaining were 0.33, 0.24, and 0.25 ng mL⁻¹, respectively. These results demonstrated a minimum adsorption/absorption of 2.7%. These data indicate that

Table 1 Ochratoxin A recovery (%) after the action of commercial peroxidase and the one obtained from rice bran at different enzymatic concentrations

Time (min)	OTA recovery (%)			
	Commercial PO (U mL ⁻¹)		PO from rice bran (U mL ⁻¹)	
	0.52	0.063	0.47	0.063
0	104.5 ± 6.5 ^a	93 ± 1 ^a	99.5 ± 7.5 ^a	91 ± 8.0 ^b
15	110.0 ± 1.5 ^a	93 ± 2 ^a	100.0 ± 5.0 ^a	102 ± 3.0 ^a
30	107.0 ± 0.5 ^a	58 ± 3 ^b	103.0 ± 1.0 ^a	79 ± 4.0 ^c
60	112.0 ± 8.0 ^a	76 ± 4 ^c	95.5 ± 2.5 ^a	80 ± 1.6 ^{bc}
300	112.5 ± 4.0 ^a	41 ± 2 ^d	100.5 ± 2.5 ^a	76 ± 0.1 ^c
1440	94.0 ± 3.0 ^b	43 ± 2 ^d	76.5 ± 11.0 ^b	59 ± 3.0 ^d

Mean ± standard deviation. Equal letters in the column indicate that there is no significant difference between the means by Tukey's test ($p < 0.05$)

the reduction of the OTA concentration observed in this study was due to the enzymatic action. Studies involving the definition of the action mechanism of PO on OTA are necessary for the biochemical characterisation of mycotoxin reduction. In addition, products obtained by enzyme activity should be investigated for their toxicity.

Enzymatic kinetics

According to the K_M and V_{max} parameters, determined by the double-reciprocal Lineweaver–Burk graph (Table 2), the commercial PO displayed a higher affinity for OTA than that extracted from rice bran (24 times higher). Thus, the rice bran-derived PO bound a relatively lower concentration of the substrate. This difference may also be due to the different degrees of purification of the enzymes.

Applicability of rice bran PO in grape juice

The PO extracted from rice bran was also intended to reduce production costs in an industrial setting, and because it was obtained from an agro-industrial co-product, it is not feasible or recommended to add another step of enzymatic inactivation in the food processing. Therefore, as pasteurisation is part of the juice processing, it was decided to verify the application in this product. The endogenous contamination was 1.98 ng mL⁻¹ in the red juice, the mean recovery of the mycotoxin was 97 and

Table 2 K_M and V_{max} for commercial peroxidase and the one obtained from rice bran

Peroxidase	K_M (μM)	V_{max} (μM min ⁻¹)
Commercial	0.27	0.015
Rice bran	6.50	0.031

93% for white and red juice, respectively, with a maximum relative standard deviation of 6%. Results of validation parameters showed that the determination coefficient was 0.9994, detection and quantification limit for the method were 0.02 and 0.03 ng mL⁻¹, guaranteeing the method quality. In view of the natural contamination results, 13 ng mL⁻¹ OTA was added to the juice. For the mycotoxin reduction test, the OTA concentrations are shown in Table 3. The values indicate a significant reduction (17%) at the OTA concentration, for the white juice, in 1440 min. For the red juice, in turn, there was no difference in the mycotoxin concentration over the time of the process.

The lower OTA reduction in juice compared to the model solution can be due to juice pH. This parameter was verified as it was believed that this could be a factor that may have affected the enzymatic activity. The juice pH was 3.3 and 3.5, for the white and red juice, respectively. From this, the evaluation of the specific activity of PO at the optimum pH (5.5) was 0.456 U mg⁻¹, while at pH 3.5 it was 0.265 U mg⁻¹ ($p < 0.05$).

Discussion

Reduction of OTA levels

The greatest reduction of the OTA concentration was noted when 0.063 U mL⁻¹ of the enzyme was added. This decrease required an 8-fold lower enzymatic concentration and 4.8-fold shorter (300 versus 1400 min) enzymatic catalysis time for the commercial PO when compared with that obtained from rice bran. The maximal decrease recorded was 59% for the commercial PO. However, a promising result of 41% was determined for the PO extracted from rice bran, especially when considering the degree of purification of this extract, as well as the low cost of the TPP technique. The fact that the reduction of OTA has presented high results with the lowest enzymatic concentration is in agreement with the enzymatic specificity,

where the substrate recognition and the higher affinity is verified when the catalyst is present in the lowest concentration.

There are few studies related to the degradation of OTA with PO. For this reason, it has been difficult to compare the results obtained in this work. Das and Mishra [33] evaluated the degrading action of PO on aflatoxin B₁. As a result, the commercial PO and two partially purified enzyme extracts from radish, with specific activities of 20 and 30 U mg⁻¹, showed 30 and 38% reduction, respectively. Therefore, the PO extracted from the rice bran had a greater reduction capacity compared to OTA, as a superior reduction (41% for the rice bran-derived PO) was demonstrated when an enzyme with less specific activity was used (0.05 U mg⁻¹). In addition, the previous authors used a high mycotoxin concentration (0.312 mg mL⁻¹), which corroborates with the higher efficiency of rice bran PO, considering trace concentrations were used in the current study.

The PO action related to the degradation or decontamination of mycotoxins was also demonstrated by Garda-Buffon [22], during submerged fermentation using the fungi *A. oryzae* and *R. oryzae*, where the reduction of deoxynivalenol (DON) mycotoxin levels was detected (74% of the reduction). The fermentation time in which the highest degradation rate of DON was observed corresponded to the higher activity of the PO produced by the microorganisms.

Feltrin et al. [31] employed the radish peroxidase enzyme (commercial standard) in DON degradation assays with tricothecene reduction of the 56% for 60 min of interaction. In another study, Feltrin et al. [34] observed the DON reduction of the 81% applying the purified peroxidase obtained from soybean meal, whereas 60% of the degradation was observed when the crude enzyme extract was applied. The reaction time for both systems, crude enzyme extract and purified, was 10 min. Gautério et al. [35] also used the enzyme peroxidase purified from rice bran and found a DON degradation of 82% in the model system. The authors [35] emphasise that oxidative activity of the enzyme may not be the only mechanism to reduce the DON concentration and that proteins may exhibit adsorption capacity due to the active groups on the surface of the protein, which may occur in substitution or in conjunction with an oxidative mechanism.

The use of peroxidase horseradish for AFLA degradation was described by Das and Mishra [36] where 60% of aflatoxin B₁ was degraded. This result is in agreement with the obtained by Zaid [37]; the peroxidase obtained from *Pseudomonas* sp. was able to degrade aflatoxin B₁ for 72 h of incubation in 87%.

Studies involving the reduction of OTA concentration by enzymes have already been evaluated by Abrunhosa, Santos and Venancio [38], Kupski, Queiroz and Badiale-Furlong [39], and Stander et al. [40], applying protease, carboxypeptidase, and lipase with reductions of 87, 78, and 90%, respectively.

Table 3 Ochratoxin A recovery after the action of peroxidase obtained from rice bran in grape juice with an initial concentration of 13 ng mL⁻¹

	OTA (ng mL ⁻¹)	
	White juice	Red juice
0	99.2 ± 2.3 ^a	93.1 ± 4.6 ^a
15	102.3 ± 3.8 ^a	96.2 ± 3.8 ^a
30	103.1 ± 6.2 ^a	93.8 ± 4.6 ^a
60	91.5 ± 1.5 ^{ab}	90.0 ± 5.4 ^a
300	102.3 ± 8.5 ^a	93.8 ± 5.4 ^a
1440	83.1 ± 2.3 ^b	97.7 ± 4.6 ^a

*Mean ± standard deviation. Equal letters in the column indicate that there is no significant difference between the means by Tukey's test ($p < 0.05$). Peroxidase activity from rice bran: 0.05 U mg⁻¹ protein

OTA concentration reduction mechanism

Gautério et al. [35] reported a reduction of 81.7% DON in a model system, using PO also purified from rice bran. The authors emphasise that they cannot affirm whether there is an oxidative action of the enzyme in the trichothecene structure. Accordingly, it raises the hypothesis that given proteins show adsorption capacity due to active groups present on the protein surface. This mechanism may occur in substitution of the oxidative action. Therefore, the OTA concentration was determined in the reactor at the beginning and after the period of contact with the denatured PO, inactivated. The final amount was found to be the same as that initially added to the system ($10 \pm 0.38 \text{ ng mL}^{-1}$). This result was expected, given the inactivation prevented the reductive action of the enzyme. In addition, the hydrolysis of PO applied in the reduction assay by pepsin confirm that the structural conformation of the protein is not related to the physical interaction, occurring OTA release if it is adsorbed/absorbed.

Enzymatic kinetics

The determination coefficients, obtained by the Lineweaver–Burk double-reciprocal plot for commercial PO and PO purified from rice bran, were 0.9994 and 0.9539, respectively. The comparatively lower value for the PO obtained from rice bran may occur due to the low purity of the enzyme, which makes it difficult to evaluate the exact mechanism. Also, it means that the protein extract possibly contains protein inhibitors of low molecular weight, which influence the binding with the substrate. The same behaviour was presented by the values documented by Abrunhosa and Venâncio [41], for an enzyme isolated and purified from *Aspergillus niger* with the capacity to hydrolyse OTA, and Suzuki et al. [42] Abrunhosa and Venâncio [40] established a K_M of 0.5 mM and V_{max} of $0.44 \mu\text{mol L}^{-1} \text{ min}^{-1}$ when the reaction was performed at 37°C , pH 7.5. Suzuki et al. [36] determined the K_M of two PO isoenzymes extracted from buckwheat seeds against several substrates, such as quercetin, *o*-dianisidine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ascorbic acid, and guaiacol. The K_M values ranged from 0.043 mM for ascorbic acid to 0.288 mM for guaiacol.

The variation between the results presented in the literature and those obtained in this study can be justified by several factors related to the quantification of the analyte. The first one concerns the concentration of OTA. The tests performed in the current study used low mycotoxin concentrations (ng mL^{-1}) because, in food, these mycotoxins are quantified at levels in the same range (around $\mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$). An example is rice which, according to Majeed et al. [43] presented an OTA concentration of $12.94 \mu\text{g kg}^{-1}$. Also, the tolerable limits stated in the Brazilian [44] and European legislation, for instance, are also described in $\mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$, which, for

rice, are 5 and $20 \mu\text{g kg}^{-1}$, respectively. Another explanation for the variations relates to the quantification/separation techniques. For the determinations performed with guaiacol, UV/Vis spectrometry was used. In contrast, for OTA, the more sensitive, high-performance liquid chromatography with fluorescence detection method was used, which can separate and quantify compounds at very low concentrations.

Applicability of rice bran PO in grape juice

There are few reports in the literature regarding the contamination of grape juice by OTA. However, the contamination of wines [9, 45] and grapes [46, 47] is widely studied. According to Varga and Kozakiewicz [48], the proportion of grape juice samples contaminated with OTA was very high, in some studies, especially for red grape juice, but lower for white juice samples. The same can be confirmed in the current work, given that the red juice displayed higher endogenous contamination than the white one. Previously, Rosa et al. [49] investigated the occurrence of OTA in wine and grape juice marketed in Rio de Janeiro (Brazil) and noticed that of 64 samples of grape juice and frozen pulp, 25% were contaminated with OTA, in concentrations ranging from 0.021 to 0.1 ng mL^{-1} . Shundo et al. [8] studied the presence of OTA in wines and grape juice marketed in the city of São Paulo (Brazil), but no mycotoxin was detected in any of the samples analysed.

The application of the enzyme as a way of reducing OTA contamination may occur before the pasteurization. In juices, this enzyme should be inactive after the process because its activity is closely linked to the disappearance of the aroma and the appearance of off-flavours in vegetal products. Besides this, the presence of phenolic compounds may have contributed to the inactivation of PO in the juice that can act as enzymatic inhibitors or as substrate. In juices, the concentration of these compounds varies, according to the species, maturation, conditions of cultivation of the grapes, and the technology applied to obtain the juice. For instance, the pulp is rich in phenolic acids, and the skin is rich in flavonoids (flavonols and anthocyanins) [50, 51]. Thus, commercially available grape juices vary widely in number and type of phenolic compounds.

Literature research on the inhibitory action of these compounds in the context of PO involves methods in which the antioxidant activity of the phenolic compounds is evaluated by their ability to inhibit the enzyme activity [52–54]. Besides, due to the high content of phenolic compounds in the red juice, it is also possible that the enzyme is acting, first, in the degradation of these compounds, if there is no enzymatic inhibition, rather than on OTA. The comparatively lower phenolic compound concentration in white juice means it was possible to verify the reduction [51].

The application of PO is, therefore, considered satisfactory in comparison to the physical and chemical methods commonly used in the degradation of OTA. However, studies are needed so that the reaction conditions of the enzyme in grape juice, or any other food product, are optimised to increase the reduction. Thus, the enzyme may be used, for example, to decrease OTA in wine, replacing the adsorbents indicated for adsorption [55, 56]. Although the authors [55] obtained a high reduction (82%), the use of adsorbents has not been well seen, due to the adsorption of some nutrients, an effect not seen when the enzyme was used because of its specificity. The same explanation applies to the study by Var et al. [57] in which the adsorption of OTA from white wine using activated carbon was studied. In that research, the authors verified that when the wine was contaminated with 5 ng mL⁻¹ OTA and treated with 1 mg mL⁻¹ activated charcoal, 87% of the available mycotoxin was adsorbed.

In general, studies aimed at evaluating the mechanism involved in reducing OTA concentration, degradation compounds, and application should be emphasised, seeking alternatives to obtain foods that show a lower risk to the population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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