Alcoholic fermentation as a strategy to mitigate pesticides and mycotoxins

Rafaela Xavier Giacomini
Federal University of Rio Grande
Eliza Rodrigues Acosta
Federal University of Rio Grande
Maristela Barnes Rodrigues Cerqueira
Federal University of Rio Grande
Ednei Gilberto Primel
Federal University of Rio Grande
Jaqueline Garda-Buffon (jaquelinebuffon@furg.br)
Federal University of Rio Grande

Research Article

Keywords: contaminant, fungicide, herbicide, mycotoxin, Saccharomyces cerevisiae, yeast

Posted Date: September 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2062216/v1

Abstract

In this study alcoholic fermentation was explored to reduce the pesticides – 2,4-dichlorophenoxyacetic acid (2,4-D) and procymidone – and the mycotoxin ochratoxin A (OTA), besides evaluating their effects on quality parameters of the process. Fermentation (at 20°C for 168 h) was conducted in synthetic must (YPD and 10 g hL^{-1} of yeast *Saccharomyces cerevisiae*) to which the following were added: 2,4-D (6.73 mg L^{-1}) and procymidone (2.24 mg L^{-1}), simultaneously (Treatment 1); and OTA (0.83 and 2.66 µg L^{-1}) (Treatments 2 and 3). The control treatment had no contaminants. All contaminated treatments showed lower ethanol production and cell viability. The highest decrease in contaminants was found after 168 h of fermentation: 22 and 65% in the cases of both pesticides while OTA decreased 52 and 58% (Treatments 2–0.83 µg L^{-1} and 3–2.66 µg L^{-1}). Decrease in procymidone and OTA due to metabolic processes in yeast cells is mainly related to peroxidase activity and glutathione production.

Introduction

Mycotoxins and pesticides are contaminants found in raw material and processed products, such as grapes and wine (Freire et al., 2020; Scariot et al., 2022). They have given cause for concern due to risks that result from human and animal exposure to them in their diets (Čepo et al., 2018; Gavahian et al., 2020). Fungicides have often been used in grape cultures to inhibit fungal growth and, consequently, mitigate losses caused by toxigenic fungi and other pathogens found in this culture (Carpinteiro et al., 2010; Gava et al., 2021). Procymidone is one of the most common fungicides applied to grapevines (Čuš et al., 2010ab; Romanazzi; Feliziani, 2014; Gava et al., 2021; Shen et al., 2021).

Procymidone is an amide fungicide which has been used to control diseases in grapes, such as gray mold, that are mainly caused by *Botrytis cinerea*. Its occurrence in grapes has become increasingly severe and has led to decrease in productivity (Shen et al., 2021). Control of the disease has been proven by several studies that report incidence of procymidone in grapes and processed products. Sala et al. (1996) found procymidone residues in white must after pressing (2.0 mg L^{-1}) and in red must after destemming and crushing (3.55 mg L^{-1}). Rose et al. (2009) showed that procymidone concentration in chardonnay grapes was higher than the maximum residue limit tolerated in Australian grapes (2.0 mg kg^{-1}) (FSANZ, 2006), since 4.5 mg kg^{-1} was reported. Čuš et al. (2010a,b) identified maximum concentrations (0.05 and 0.13 mg L^{-1}) of the fungicide in wine, respectively. Besides, other studies have focused on decrease of the fungicide in stages of the vinification process, such as filtration and clarification (Doulia et al., 2016, 2017).

Another problem that the vitiviniculture sector has faced is cross-contamination caused by pesticides whose origins are other agricultural crops, such as rice and soybean, which also cause negative impact on grapevines, mainly the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Rossouw et al., 2019). Its effect may be found several kilometers from the target, mainly when there are prevailing winds when it is applied (Felsot et al. 2010).

Frequent use of active ingredients in vineyards (Medina et al., 2007; Costa et al., 2019), too much irrigation, humid climate and fine grape skin enable colonization by toxigenic fungal species that may produce mycotoxins under stress conditions (Csutorás et al., 2013; González-Domínguez et al., 2019; Torović et al., 2020). Due to their high toxicity and risks to health, mycotoxins have been investigated in raw material and food (Medina et al., 2007; Costa et al., 2019; Kochman et al., 2021). The most important mycotoxin in wine is ochratoxin A (OTA), which is

produced by different fungal species that belong to both genera *Penicillium* and *Aspergillus* (Čepo et al., 2018; Kersh et al., 2022). Taking into consideration their carcinogenic properties (IARC, 1993), their levels in wine must be strictly controlled, mainly regarding OTA intake in the daily diet, i. e., from 10 to 13% of the intake may originate in wine (Miraglia & Brera, 2002). In the legislation, maximum OTA level in wine is 2 μg L^{-1} (ANVISA, 2013; EC, 2006) while weekly OTA intake was established at 0.12 μg kg^{-1} of body weight (EFSA, 2010).

Regarding forms of detoxification of contaminants, alcoholic fermentation with *Saccharomyces cerevisiae* along with malolactic fermentation with lactic bacteria has stood out as a promising biological method that decreases concentrations of mycotoxins and pesticides (Doulia et al., 2016, 2017; Torović et al., 2020; Boeira et al., 2021; Scariot et al., 2022). Its potential may be related to a mechanism of defense against oxidative stress caused by contaminants since it may activate protection molecules and activities of enzymes, such as reduced glutathione (GSH) and peroxidase (PO) (Dong et al., 2007; Halliwell & Gutteridge, 2015; Boeira et al., 2021; Junior et al., 2021). Therefore, studies of mitigation of mycotoxins and pesticides by alcoholic fermentation with yeasts should be carried out. In this study aimed alcoholic fermentation was explored to reduce the pesticides – 2,4-dichlorophenoxyacetic acid (2,4-D) and procymidone – and the mycotoxin ochratoxin A (OTA)

Materials And Methods

Standard solutions of 2,4-D, procymidone and OTA

Standard solutions of 2,4-D, procymidone and OTA (purity > 98%) were purchased from Sigma-Aldrich Brasil. Stocks solutions of the herbicide 2,4-D and the fungicide procymidone (1000 μg mL^{-1}) were prepared in acetonitrile (MeCN). The stock solution of OTA (100 μg mL^{-1}) was prepared in benzene:MeCN (98:2 v v^{-1}). Concentration of the OTA solution was confirmed by a UV-Vis spectrophotometer. Wavelength of maximum OTA absorption was 333 nm while molar absorptivity was 5550 L cm^{-1} mol^{-1} in benzene:acetic acid (99:1, v v^{-1}) (AOAC, 2000).

Synthetic must and inoculum used in alcoholic fermentation

Synthetic must in alcoholic fermentation (YPD) was composed of glucose (220 g L^{-1}), tartaric acid (4 g L^{-1}), yeast extract (10 g L^{-1}) and peptone (20 g L^{-1}) (Zhang et al., 2007). The yeast *Torulaspora delbrueckii (Zymaflore® Alpha) at* 3 g hL^{-1} *was associated with S. cerevisiae* (Zymaflore® Xpure) (10 g hL^{-1}) reconstituted with Superstart® Rouge (yeast preparation product which is rich in ergosterol) (10 g hL^{-1}), in agreement with the manufacturer's instructions, to compose the inoculum employed in fermentation. The acid-lactic bacterium *Oenococcus oeni* (1 g hL^{-1}) was added to carry out malolactic fermentation.

Conditions of alcoholic fermentation

Submerged cultivation was conducted in 500-ml Erlenmeyer flasks with 240 mL synthetic must and 10 mL inoculum. Fermentation was carried out at 26°C for 168 h. One g hL^{-1} of the acid-lactic bacterium *O. oeni* was added 48 hours after the beginning of alcoholic fermentation.

Decrease in contaminant concentrations and their effects on alcoholic fermentation were evaluated by adding 2,4-D, procymidone and OTA to the flasks. Both pesticides were evaluated simultaneously, i. e., 2,4-D at 6.73 mg L^{-1} and procymidone at 2.24 mg L^{-1} (Treatment 1) were added at the same time. Both concentrations

were found in a study of the occurrence of contaminants in southern Brazil (unpublished data). This procedure was also used in relation to both OTA concentrations in the must: 0.84 μg L^{-1} (Treatment 2) was the concentration found by the study of its occurrence while 2.66 μg L^{-1} (Treatment 3) was the maximum limit established by the Brazilian legislation and the European one in the case of grapes (2.0 μg kg^{-1}).

Solutions of the mycotoxin and pesticides were added to the flask and followed by solvent evaporation under nitrogen flow. Afterwards, synthetic must was added to them and homogenized for 10 min. Then, the inoculum was also added. The control treatment was carried out with no contaminant. Besides, a treatment with neither yeasts nor contaminants was conducted to evaluate the fermentation medium. Aliquots were aseptically collected every 24 h to monitor PO activity and concentrations of the following: cell, reducing sugar, GSH, ethanol, 2,4-D, procymidone and OTA.

Analytical determinations

Biomass concentration was quantified by a spectrophotometer (FEMTO Cirrus-80) by optical density (OD) at 600 nm. OD was converted into dry biomass with the use of the yeast standard curve by the gravimetric method (dry weight). Cells were dried at 60°C up to constant weight (Chang et al., 2018).

Cell viability (%) was carried out by the Trypan blue dye exclusion assay (0.2%). The dye (20 μL) was added to a sample aliquot (500 μL). After a minute, a drop of the solution was placed in a Neubauer chamber and viable and non-viable cells were counted in a microscope. Calculation was estimated by the relation between viable cells and non-viable ones (Strober, 2015).

Maximum growth rate (μ_{max}) resulted from exponential regression applied to the phase of logarithmic growth (Hamidi-Esfahani et al., 2007).

Intracellular GSH was quantified by the colorimetric method determined by Owens and Belcher (1965) with the use of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) evaluated by a spectrophotometer (FEMTO Cirrus-80) at 412 nm. GSH concentration (mg L^{-1}) was calculated with the use of the L-GSH standard curve.

PO activity was evaluated by a spectrophotometer at 470 nm in agreement with Garda-Buffon et al. (2011), who use hydrogen peroxide and guaiacol as the substrate. An enzyme unit (U) was defined as the amount of enzyme which is capable of oxidizing or hydrolyzing 1 μmol substrate per min (Feltrin et al., 2017). Molar absorptivity of guaiacol ($_{470}$) de 26600/(M cm) was considered.

Reducing sugars were determined by the spectrophotometric method of 3,5 Dinitrosalicylic acid (DNS) (Miller, 1959), which uses a glucose standard curve.

Ethanol was quantified in agreement with Klarić et al. (2015). A gas chromatograph (CG-2010 Shimadzu) equipped with a split/splitless and flame ionization detector (FID) and a Crossbond column (diphenyl dimethyl polysiloxane), 30 m in length, 0.25 mm in inner diameter and 0.23 μm in thickness (75% phenyl-methylpolysiloxane film), was used. Manual injection volume was 1 μL of fermented medium, initial temperature was 230°C, column pressure was 2.33 psi and nitrogen carrier gas flow was 0.5 mL min^{-1}. Average velocity of nitrogen gas was 10 cm s^{-1}. Oven temperature was kept at 26°C for 7 min and then increased to 50°C at 1°C min^{-1} up to 200 °C (at 15°C min^{-1}) for 4 min. FID temperature was 250°C while nitrogen carrier gas flow was 25 mL min^{-}

^{1}. Ethanol was determined by the standard curve of ethanol diluted in ultrapure water and results were expressed as percentages.

Extraction and quantification of pesticides in the fermented medium

The herbicide 2,4-D and the fungicide procymidone were extracted from fermented media by the QuEChERS method described by Payá et al. (2007), with modifications, i. e., citrate salts sesquihydrate and dihydrate were eliminated. Extraction was carried out by MeCN and the extract was cleaned by both salts magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl). The resulting extract was used for identifying and quantifying the pesticides by LC-DAD and GC-MS.

To separate the herbicide 2,4-D, the analytical chromatographic column Supelco® - Kromasil C18 (5 µm and 150 mm x 4.6 mm) was used in a Shimadzu Liquid Chromatograph (Kyoto, Japan) with a Diode Array Detector (LC–DAD). The mobile phase consisted of MeCN and acidified Mili-Q water (aqueous phosphoric acid solution 1:1, v v^{-1}, pH: 3.0), 52:48 (v v^{-1}). Flow rate was 0.8 mL min^{-1}, temperature was 25 ºC and retention time was 5.52 min. The wavelength used for identifying 2,4-D was 220.3 nm. Injection volume was 20 µL, as proposed by Caldas et al. (2009).

To separate the fungicide procymidone, the RTX-5MS column (30m x 0.25 mm ID x 0.25 µm) was used in a Shimadzu gas chromatograph QP2010 Plus (Kyoto, Japan) equipped with an autosampler (AOC-20i) coupled to a mass spectrometer with a quadrupole mass filter (GC–MS). Injection volume was 1 µL, as described by Barbosa et al. (2020).

Extraction and quantification of OTA

OTA was extracted from the fermented medium by the QuEChERS method described by Fernandes et al. (2013), with the use of acidified MeCN (1% acetic acid) and partition with addition of a mix of salts: $MgSO_4$, NaCl, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate. Resulting dry extract was resuspended in the mobile phase (60% MeCN, 40% Mili-Q water acidified with 1% acetic acid), followed by chromatographic injection (20 µL).

Separation, identification and quantification required the analytical chromatographic column Supelco® - Kromasil C18 (5 µm and 150 mm x 4.6 mm) at 25 ºC in a Shimadzu Liquid Chromatograph (Kyoto, Japan) with a fluorescence detector (LC-FL). Wavelengths were 333 nm and 460 nm for excitation and emission, respectively. The mobile phase consisted of MeCN:water acidified with 1% acetic acid (60:40, v v^{-1}), flow rate was 0.8 mL min^{-1} and injection volume was 20 µL, as described by Garcia et al. (2020).

Reduction in contaminant concentrations

Reduction of contaminants in alcoholic fermentation was evaluated by Eq. (1), where $Q0_i$ is the amount of residue of contaminants at the beginning of the process and Q_i is the amount of residue of contaminants in the processed product (Doulia et al., 2017; Hou et al., 2020).

$$\text{Reduction } (\%) = \left[\frac{\bar{Q}0_i - \bar{Q}_i}{\bar{Q}0_i} \right] * 100$$

1

Redox-specific degradation and velocity of degradation in relation to GSH and PO of contaminants in alcoholic fermentation

Estimate of the redox-specific degradation (RSD) of contaminants in fermented medium was based on GSH concentration and PO activity in yeast cells by Eq. (2), where PDE (%) is the percentage of degradation of the contaminant determined in the fermented medium, GSH is GSH concentration (mg mL^{-1}), PO is peroxidase activity (U mL^{-1}) and t is sample collection time in the fermentation process (h).

RSD (%deg/$_{GSH}$*U_{PO}*h) = $\left[\frac{PDE(\%)}{GSH*PO*t} \right]$ (2)

Estimate of velocity of degradation in relation to GSH and PO (VDE_{GSH*PO}) was based on the degraded mass of the contaminant, GSH concentration, PO activity and fermentation time (t), expressed as Eq. 3 (Garda-Buffon et al., 2011).

VDE_{GSH*PO} (µg/GSH*U*h) = $\left[\frac{\text{mass of contaminant}}{[GSH]*PO*t} \right]$ (3)

Statistical analysis

Statistical analysis of data was conducted by the analysis of variance (ANOVA), followed by the Tukey's test at 5% significance ($p < 0.05$), by the Action Stat® 3.0 software program (Estatcamp & DIGUP, 2016). Responses of alcoholic fermentation with and without contaminants were biomass concentration (mg mL^{-1}), cell viability (%), PO specific activity (U mL^{-1}), GSH production (mg L^{-1}), reducing sugars (mg mL^{-1}) and ethanol (%).

The set of data on analyses in triplicate of VDE_{GSH*PO} (µg/GSH*U*h), RSD (%deg/$_{GSH}$*U_{PO}*h), GSH production (mg L^{-1}), PO specific activity (U mL^{-1}) and reduction of contaminants (%) was evaluated by the principal component analysis (PCA) by the PAST 2.04 software program (Hammer et al., 2001).

Results And Discussion

Effects of contaminants on alcoholic fermentation

Biochemical and kinetic characterization of alcoholic fermentation: cell concentration and viability, GSH, PO, reducing sugar, ethanol and μ_{max}

It is fundamental to follow cell concentration, GSH production, PO activity, reducing sugar concentration, ethanol production and cell viability to evaluate effects of mycotoxins and pesticides on alcoholic fermentation. Therefore, these parameters were monitored daily in the 168-h fermentation (Table 1). Regarding cell concentration, exponential growth was found up to 72 h of fermentation in all culture media (Tables 1 and 2). After 72 h, cell concentrations decreased differently as the result of treatments. The highest decrease was found

after 168 h in treatments exposed to OTA. Both Treatments 2 and 3 (exposed to OTA) led to decrease in cell concentrations that ranged from 28 to 40% after 168 h, by comparison with the other treatments (Table 1). Both 2,4-D and procymidone exhibited no significant difference from the control treatment at the end of alcoholic fermentation, after 168 h (Table 1). Maximum growth rate (μ_{max}) also decreased in contaminated treatments, i. e., 9 and 12.5% in treatments exposed to OTA and 1.0% in the treatment exposed to pesticides, by comparison with the control treatment (Table 2). Besides, 24 h after fermentation, there was decrease in cell viability in all contaminated treatments by comparison with the control treatment (Table 1) while 168 h after fermentation, there was decrease of 15% in cell viability in Treatment 1 and 4 and 7% in Treatments 2 and 3, respectively, by comparison with the control treatment. Simultaneous addition of pesticides to culture media decreased cell viability significantly.

Insert Table 1

Insert Table 2

The mechanism of action of pesticides in cells is distinct and not very clear, depending on the organism (Owsiak et al., 2021). Herbicides, including 2,4-D, have been known for affecting biological systems negatively (Ritcharoon et al., 2020). Since negative effects affect gene expression, they trigger responses to stress and lead to interruption of cell cycle control, of immune responses and of DNA repair (Bharadwaj et al., 2005).

Toxicity of 2,4-D towards yeast cells is mainly due to the activity of the non-dissociated form (Cabral et al., 2003). It suggests that the lipid bilayer in plasma membranes is one of the biological targets of the herbicide; it may be either due to direct interaction between this highly lipophilic form and membrane lipids, thus affecting spatial organization of membranes (Heipieper et al., 1994), or due to lipid peroxidation as the consequence of its activity as a pro-oxidant agent (Teixeira et al., 2004). On the other hand, fungicides aim at disruption of integrity of cell membranes and cell walls of fungi. Toxic effects of fungicides may not only result in instability in cell walls, changes in osmolarity and production of reactive oxygen species (ROS) (Hayes et al., 2014), but also induce oxidative stress as the result of accumulation of free radicals in cells (Grosicka-Maciąg, 2011). Different activities of both pesticides classes may have influenced high decrease in viability found in Treatment 1.

Teixeira et al. (2004) showed that exposure of *S. cerevisiae* cells to 2,4-D at 0.45 and 0.65 mM induces a period of growth latency in which the cell population loses viability, followed by resumption of exponential growth of the adapted population. It shows that toxic compounds in alcoholic fermentation influence concentration and/or cell viability in yeasts and may cause cell death induced by chemical stress of contaminants.

When yeasts are subject to stress conditions induced by intrinsic and extrinsic factors, they increase their energy consumption, which leads to changes in metabolism and, consequently, accumulation of protection molecules and activation of enzyme systems, such as GSH and PO (Rollini & Manzoni, 2006; Dong et al., 2007; Boeira et al., 2021). These molecules are involved in cell physiological processes that are related to protection against oxidative stress and may also affect cell detoxification (Wang et al., 2014).

Increase in PO activity and in GSH concentration was different in every treatment. The control treatment exhibited the highest enzyme activity after 72 h of fermentation; it was 15% higher than the treatment exposed to pesticides and 18% lower than treatments exposed to OTA. However, the highest concentration of GSH was found after 96 h;

it was 93% higher than the treatment exposed to pesticides and 53 and 13% higher than Treatments 2 and 3 exposed to OTA, respectively. Production of these molecules results from metabolic pathways that are typical of alcoholic fermentation (Boeira et al., 2021; Scariot et al., 2022) but, in Treatment 1, the opposite of the control treatment was observed. In 72 h of fermentation, there was increase in GSH production, which was 7% higher that the control treatment and 9 and 18.5% lower that Treatments 2 and 3. The highest PO activity was found after 96 h, i. e., 20% higher that the control treatment and 18% lower than Treatments 2 and 3.

In treatments with OTA, PO activity in Treatment 2 was higher after 168 h by comparison with both Treatment 1 (27%) and the control treatment (52%). However, increase in GSH concentration agreed with the one of the contaminant after 48 h in Treatment 3 (2.66 μg L^{-1}), the highest concentration in the shortest fermentation time and after 72 h in Treatment 2 (0.84 μg L^{-1}). They were 33 and 90% higher than the control treatment and 16 and 9% higher than Treatment 1, respectively.

These differences are strongly related to contaminants added to the media (Viegas et al., 2005). Both treatments exposed to OTA (Treatments 2 and 3) exhibited the highest PO activities after 168 h (11.47 U mL^{-1}), by comparison with the other treatments (Table 1). It is due to the direct correlation among the mycotoxin, its toxicity and the potential of yeast cells to produce specific enzymes that act on the maintenance of metabolic activity of microorganisms (Boeira et al., 2021). In contaminated treatments, increase in GSH production took place before the highest PO activity was found. This metabolic alteration may show that cells, after 48 h of culture, develop mechanisms of biodegradation of compounds that are oxidative to yeasts and may be associated with degradation of toxic compounds, a metabolic pathway induced by the contaminants throughout the culture (Garda-Buffon & Badiale-Furlong, 2010).

Contaminants lead to generation of ROS and convert GSH, which is the most abundant antioxidant molecule in the intracellular medium, into oxidized glutathione (GSSG), thus, decreasing toxicity in the medium (Lu, 2013). Antioxidant activity of GSH is mostly carried out by reactions catalyzed by GSH peroxidase (GPx), which reduce hydrogen peroxide and lipid peroxide as GSH is oxidized to GSSG. Regeneration of GSH from GSSG takes place through glutathione reductase. Thus, to balance redox reactions, cells may induce glutathione reductase activity in order to increase GSH and the relation GSH/GSSG (Bitani et al., 2022).

This study is extremely important since data on activities of protection molecules and enzyme systems, such as GSH and PO, were related to different toxic compounds. Thus, it shows that both pesticides and the mycotoxin respond differently to production of molecules GSH and PO related to the fermentation period connected with molecule conversion to keep redox balance of cells.

Reducing sugar and ethanol

All contaminated treatments in culture media affected ethanol production (% v v^{-1}) by *S. cerevisiae* (Table 1). The control treatment, after 72 h of fermentation, exhibited 13% of ethanol production while Treatments 1 (exposed to pesticides), 2 and 3 (exposed to the mycotoxin) exhibited lower ethanol production, i. e., 12.9, 10.6 and 9%, while reduction was 0.8, 23.0 and 44%, respectively.

The end of alcoholic fermentation was confirmed by low levels of reducing sugars, between 1.7 and 2.3 mg mL^{-1} after 168 h (Table 1). Briz-Cid et al. (2018) evaluated the influence of four treatments with fungicides (metrafenone, boscalid + kresoxim-methyl, fenhexamid and mepanipyrim) on Tempranillo wine. The authors

found that fermentation kinetics is influenced not only by grape composition but also by fungicides. Grapes treated with a mix of boscalid (200 mg mL^{-1}) and kresoxim-methyl (100 mg mL^{-1}) exhibited delay at the beginning of alcoholic fermentation but ended together with the others. Therefore, high concentrations of contaminants (mycotoxins and pesticides) in grapes may result in slow fermentation or paralyze it and, consequently, harm ethanol production (Kłosowski et al., 2010).

Ethanol concentration is also related to GSH production (Table 1) (Wen et al., 2005; Margalef-Català et al., 2017), which may be classified into three phases throughout alcoholic fermentation. In the first phase, glucose levels decrease gradually while concentrations of ethanol and glutathione increase. In the second phase, ethanol is used as the carbon source for cell growth and glutathione synthesis. In the third phase, both glucose and ethanol are consumed and cells stop multiplying (Wen et al., 2005). Table 1 shows that, between 48 and 72 h of fermentation, increase in ethanol levels may be related to increase in GSH production in treatments exposed to contaminants, by comparison with the control treatment, an effect that was not observed in the control treatment, when GSH only increased after 96 h of fermentation.

Dong et al. (2007) stated that cell stress – in this case, caused by contaminants – influences increase in energy consumption by yeasts and leads to changes in metabolism and accumulation of some protection molecules, such as GSH. Although some studies show initial inhibition caused by contaminants, yeasts outperform them and resume fermentation (Briz-Cid et al., 2018; Scariot et al., 2022). This fact is confirmed by sugar consumption and ethanol production, markers that are fundamental to control fermentation (Samphao et al., 2018; Hu et al., 2022; Zhang et al., 2022).

Reduction of contaminants in alcoholic fermentation

The culture medium exposed to pesticides 2,4-D and procymidone at 6.73 mg L^{-1} (2,4-D) and 2.24 mg L^{-1} (procymidone) exhibited decrease of 22 and 65%, respectively, after 168 h of fermentation (Figure 1). Culture media exposed to OTA at 0.83 µg L^{-1} and 2.66 µg L^{-1} exhibited decrease of 52 and 58%, respectively, after 168 h of fermentation (Figure 1).

After 24 h of fermentation, decrease in contaminants in culture media are simultaneous to increase in PO activity (Tables 1 and 3), the period in which the yeast activates metabolic pathways to decrease toxicity in the culture medium, thus, preserving cells in the fermentation process (Garda-Buffon & Badiale-Furlong, 2010). Therefore, the highest RSD and VDE_{GSH*PO} were found after 24 h of alcoholic fermentation in the cases of 2,4-D, procymidone and OTA in all treatments (Table 3). The highest percentage of procymidone degradation was 61.18%$deg/_{GSH}*U_{PO}*h$ at the velocity of 1.320 µg/GSH*U*h (Table 3) and, consequently, the highest reduction (50%) (Figure 1), which confirms that compound oxidoreduction may be related to the potential that yeast cells have to produce specific molecules (GSH and PO) to aim at maintaining metabolic activity of the yeast (Lash, 2005).

Insert Figure 1

Insert Table 3

The highest decrease in OTA and pesticides took place at the end of alcoholic fermentation (168 h) (Figure 1). Besides being related to GSH and PO activity, decrease may also be related to the adsorptive capacity of *S.*

cerevisiae (Meca et al., 2010). The adsorption process on the yeast wall is related to mannoproteins which are capable of bounding to pesticides and mycotoxins (Čuš et al., 2010b; Meca et al., 2010; Freire et al., 2020). Some studies suggest that the adsorption process is easily observed when mannoproteins are released in the first week after the end of alcoholic fermentation, mainly when yeast lees are homogenized in wine at the end of the fermentation process (*battonage*, the stirring technique), which ends up becoming a measurer of the potential yeasts have to mitigate contaminants (González-Rodríguez et al., 2009; Čuš et al., 2010b).

Efficacy of detoxification of different toxic compounds, such as pesticides and mycotoxins by yeast activity, depends on several factors, such as the type of strain, and concentration and incubation time of compounds, throughout the process (Yousefi & Khorshidian; Mortazavian, 2021). High doses of toxic compounds exert negative influence on both *S. cerevisiae* growth and alcoholic fermentation (Li et al., 2012; Scariot et al., 2022).

To better understand the relation between decrease in contaminants in alcoholic fermentation, the PCA was carried out (Figure 2) to focus on two regions. The first component (PC 1) explains 49.5% of total variance. Therefore, about 50% of information found in the five variables of the database may be encompassed by this component. The second component (PC 2) explains 33.5% of total variance. Thus, 83% of data variance is explained by only two components.

The region delimited by a circle accounts for variables VDE_{GSH*PO} and RSD of contaminants. The Pearson correlation shows positive and significant relation ($R = 0.66$, $p = 3.88 \times 10^{-4}$) between both variables. It means that, after 24 h of fermentation, *S. cerevisiae* activates metabolic pathways to decrease toxicity in the culture medium, which is mainly related to increase in VDE_{GSH*PO} and RSD of contaminants from this period of fermentation on (Table 3). It highlights the fact that alcoholic fermentation mitigates contamination caused by pesticides and mycotoxins in treatments.

The region represented by a square accounts for variables GSH and PO. Positive and significant correlation ($R = 0.78$, $p = 5.99 \times 10^{-6}$) was also found between both variables. It shows that Treatments 2 and 3 exposed to OTA exhibited high activation of those molecules (Table 1). It may be explained by OTA toxicity, which makes yeast cells produce defense systems to keep their metabolic activity.

Positive and significant correlation between reduction of contaminant and PO activity ($R = 0.65$, $p = 0.0005$), asterisked in the graph, should also be highlighted. It shows that, the lower the compound toxicity, the more it decreases, i. e., procymidone, the compound that has the lowest toxicity in this study, exhibited the highest decrease (65%), by comparison with the other contaminants (Figure 1), mainly related to PO activity. On the other hand, 2,4-D, which was classified into Group 2B as likely to be carcinogenic to humans (IARC, 2018), exhibited the lowest decrease (22%). It is the only contaminant that is not related to the variables shown by the PCA. This fact leads to the need for further studies to investigate other relations between enzyme pathways and 2,4-D degradation. Its mitigation by decreasing adsorption though cell walls of yeasts – unlike the other contaminants – may take place but other metabolic processes in their cells should be investigated so as to mitigate human exposure to these contaminants which are often found in grapes and wine.

Insert Figure 2

Conclusion

Both pesticides and the mycotoxin influenced biochemical characteristics in alcoholic fermentation. Contamination affected cell viability (the lowest ranged from 4 to 18%), which may be related to the energy spent by the yeast to activate metabolic pathways in detoxification. Decrease in OTA, 2,4-D and procymidone took place after 24 h of fermentation and the highest index was found at the end of the alcoholic fermentation (168 h). Decrease in procymidone and OTA due to metabolic processes in yeast cells is mainly related to PO activity and GSH production.

Declarations

Author Contribution: Rafaela Xavier Giacomini: conceptualization, methodology, software, validation, data curation, writing. Eliza Rodrigues Acosta: methodology, formal analysis. Maristela Barnes Rodrigues Cerqueira: validation, formal analysis. Ednei Gilberto Primel: resources, funding acquisition. Jaqueline Garda-Buffon: conceptualization, project administration, reviewing and editing, supervision, funding acquisition.

Acknowledgments

The authors are gratefull to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code [001], Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Federal University of Rio Grande (FURG) for financial support and infrastructure.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) [001]; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [308565/2021-5].

Competing Interests/Conflict of Interest: The authors declare no competing interests.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. ANVISA (Agência Nacional de Vigilância Sanitária). (2011). *Resolução da diretoria colegiada - RDC nº 7, de 18 de fevereiro de 2011 limites máximos tolerados (LMT) para micotoxinas em alimentos*. Diário Oficial da União, 37: Brazil. Retrieved September 05, 2022, from https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/resolucao-rdc-no-7-de-18-de-fevereiro-de-2011.pdf
2. AOAC (Association of Official Analytical Chemists). 2000. *Official Methods of Analysis*. Natural Toxins, Chap. 49, p. 4.
3. Barbosa, S. C., Cerqueira, M. B, Primel, E. G., Furlong, E. B., & Kupski. L. (2020). Validation of QuEChERS and GC-MS for pesticides determination in rice samples. *Revista Mundi Engenharia, Tecnologia e Gestão*, *5*(7), 280–01, 280–16. https://doi.org/10.21575/25254782rmetg2020vol5n71107
4. Bharadwaj, L., Dhami, K., Schneberger, D., Stevens, M., Renaud, C., & Ali, A. (2005). Altered gene expression in human hepatoma HepG2 cells exposed to low-level 2, 4-dichlorophenoxyacetic acid and potassium nitrate.

Toxicology in vitro, *19*(5), 603–619. https://doi.org/10.1016/j.tiv.2005.03.011

5. Binati, R. L., Larini, I., Salvetti, E., & Torriani, S. (2022). Glutathione production by non-Saccharomyces yeasts and its impact on winemaking: A review. *Food Research International*, *156*, 111333. https://doi-org.ez40.periodicos.capes.gov.br/10.1016/j.foodres.2022.111333
6. Boeira, C. Z., de Carvalho Silvello, M. A., Remedi, R. D., Feltrin, A. C. P., Santos, L. O., & Garda-Buffon, J. (2021). Mitigation of nivalenol using alcoholic fermentation and magnetic field application. *Food Chemistry*, *340*, 127935. https://doi.org/10.1016/j.foodchem.2020.127935
7. Briz-Cid, N., Castro-Sobrino, L., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J. (2018). Fungicide residues affect the sensory properties and flavonoid composition of red wine. *Journal of Food Composition and Analysis*, *66*, 185–192. https://doi.org/10.1016/j.jfca.2017.12.021
8. Cabral, M. G., Viegas, C. A., Teixeira, M. C., & Sa-Correia, I. (2003). Toxicity of chlorinated phenoxyacetic acid herbicides in the experimental eukaryotic model Saccharomyces cerevisiae: role of pH and of growth phase and size of the yeast cell population. *Chemosphere*, *51*(1), 47–54. https://doi.org/10.1016/S0045-6535(02)00614-8
9. Caldas, S. S., Demoliner, A., & Primel, E. G. (2009). Validation of a method using solid phase extraction and liquid chromatography for the determination of pesticide residues in groundwaters. *Journal of the Brazilian Chemical Society*, *20*, 125–132. https://doi.org/10.1590/S0103-50532009000100020
10. Carpinteiro, I., Ramil, M., Rodríguez, I., & Cela, R. (2010). Determination of fungicides in wine by mixed-mode solid phase extraction and liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography A*, *1217*(48), 7484–7492. https://doi.org/10.1016/j.chroma.2010.09.080
11. Čepo, D. V., Pelajić, M., Vrček, I. V., Krivohlavek, A., Žuntar, I., & Karoglan, M. (2018). Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. *Food Chemistry*, *246*, 394–403. https://doi.org/10.1016/j.foodchem.2017.10.133
12. Chang, Y. H., Chang, K. S., Chen, C. Y., Hsu, C. L., Chang, T. C., & Jang, H. D. (2018). Enhancement of the efficiency of bioethanol production by Saccharomyces cerevisiae via gradually batch-wise and fed-batch increasing the glucose concentration. *Fermentation*, *4*(2), 45. https://doi.org/10.3390/fermentation4020045
13. Costa, C. L. D. A., Cerqueira, M. B. R., & Garda-Buffon, J. (2019). Kresoxim-methyl and famoxadone as activators of toxigenic potential of *Aspergillus carbonarius*. *Food Additives* & Contaminants: Part A, *36*, 1860–1870. https://doi.org/10.1080/19440049.2019.1670869
14. Csutorás, C., Rácz, L., Rácz, K., Fűtő, P., Forgó, P., & Kiss, A. (2013). Monitoring of ochratoxin A during the fermentation of different wines by applying high toxin concentrations. *Microchemical Journal*, *107*, 182–184. https://doi.org/10.1016/j.microc.2012.07.001
15. Čuš, F., Česnik, H. B., Bolta, Š. V., & Gregorčič, A. (2010a). Pesticide residues and microbiological quality of bottled wines. *Food Control*, *21*, 150–154. https://doi.org/10.1016/j.foodcont.2009.04.010
16. Čuš, F., Česnik, H. B., Bolta, Š. V., & Gregorčič, A. (2010b). Pesticide residues in grapes and during vinification process. *Food Control*, *21*, 1512–1518. https://doi.org/10.1016/j.foodcont.2010.04.024
17. Dong, Y., Yang, Q., Jia, S., & Qiao, C. (2007). Effects of high pressure on the accumulation of trehalose and glutathione in the *Saccharomyces cerevisiae* cells. *Biochemical Engineering Journal*, *37*(2), 226–230. https://doi.org/10.1016/j.bej.2007.04.004
18. Doulia, D. S., Anagnos, E. K., Liapis, K. S., & Klimentzos, D. A. (2016). Removal of pesticides from white and red wines by microfiltration. *Journal of Hazardous Materials*, *317*, 135–146.

https://doi.org/10.1016/j.jhazmat.2016.05.054

19. Doulia, D. S., Anagnos, E. K., Liapis, K. S., & Klimentzos, D. A. (2017). Effect of clarification process on the removal of pesticide residues in white wine. *Food Control*, *72*, 134–144. https://doi.org/10.1016/j.foodcont.2016.07.035
20. EC (European Commission). (2006). *Commission Regulation No 1881/2006 of 19 December 2006, setting maximum levels of certain contaminants in foodstuffs*. Official Journal of European Communities L, 364/5: European Union. Retrieved September 5, 2022, from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006R1881
21. EFSA (European Food Safety Authority). (2010). Statement on recent scientific information on the toxicity of Ochratoxin A. *EFSA Journal*, *8*(6), 1626. https://doi.org/10.2903/j.efsa.2010.1626
22. Estatcamp, & DIGUP. (2016). *Action Stat*. São Carlos-SP, Brazil. Retrieved September 05, 2022, from www.portalaction.com.br
23. FSANZ (Food Standards Australia New Zealand). (2006). Web address: https://www.foodstandards.gov.au/code/applications/Documents/FAR_A559_MRLs_Apr_May_June_05_.pdf
24. Feltrin, A. C. P., Garcia, S. D. O., Caldas, S. S., Primel, E. G., Badiale-Furlong, E., & Garda-Buffon, J. (2017). Characterization and application of the enzyme peroxidase to the degradation of the mycotoxin DON. *Journal of Environmental Science and Health, Part B*, *52*(10), 777–783. https://doi.org/10.1080/03601234.2017.1356672
25. Fernandes, P. J., Barros, N., & Câmara, J. S. (2013). A survey of the occurrence of ochratoxin A in Madeira wines based on a modified QuEChERS extraction procedure combined with liquid chromatography–triple quadrupole tandem mass spectrometry. *Food Research International*, *54*(1), 293–301. https://doi.org/10.1016/j.foodres.2013.07.020
26. Freire, L., Braga, P. A., Furtado, M. M., Delafiori, J., Dias-Audibert, F. L., Pereira, G. E., Reyes, F. G., Catharino, R. R., & Sant'Ana, A. S. (2020). From grape to wine: Fate of ochratoxin A during red, rose, and white winemaking process and the presence of ochratoxin derivatives in the final products. *Food Control*, *113*, 107167. https://doi.org/10.1016/j.foodcont.2020.107167
27. Felsot, A. S., Unsworth, J. B., Linders, J. B., Roberts, G., Rautman, D., Harris, C., & Carazo, E. (2010). Agrochemical spray drift; assessment and mitigation—A review. *Journal of Environmental Science and Health Part B*, *46*(1), 1–23. https://doi.org/10.1080/03601234.2010.515161
28. Garcia, S. O., Sibaja, K. V. M., Nogueira, W. V., Feltrin, A. C. P., Pinheiro, D. F. A., Cerqueira, M. B. R., Furlong, E. B., & Garda-Buffon J. (2020). Peroxidase as a simultaneous degradation agent of ochratoxin A and zearalenone applied to model solution and beer. *Food Research International*, *131*, 109039. https://doi.org/10.1016/j.foodres.2020.109039
29. Garda-Buffon, J., & Badiale-Furlong, E. (2010). Kinetics of deoxynivalenol degradation by Aspergillus oryzae and Rhizopus oryzae in submerged fermentation. *Journal of the Brazilian Chemical Society*, *21*(4), 710–714. https://doi.org/10.1590/S0103-50532010000400018
30. Garda-Buffon, J., Kupski, L., & Badiale-Furlong, E. (2011). Deoxynivalenol (DON) degradation and peroxidase enzyme activity in submerged fermentation. *Food Science and Technology*, *31*, 198–203. https://doi.org/10.1590/S0101-20612011000100030
31. Gava, A., Emer, C. D., Ficagna, E., Fernandes de Andrade, S., & Fuentefria, A. M. (2021). Occurrence and impact of fungicides residues on fermentation during wine production–A review. *Food Additives & Contaminants:*

Part A, *38*(6), 943–961. https://doi.org/10.1080/19440049.2021.1894357

32. Gavahian, M., Pallares, N., Al Khawli, F., Ferrer, E., & Barba, F. J. (2020). Recent advances in the application of innovative food processing technologies for mycotoxins and pesticide reduction in foods. *Trends in Food Science & Technology*, *106*, 209–218. https://doi.org/10.1016/j.tifs.2020.09.018.
33. González-Domínguez, E., Fedele, G., Languasco, L., & Rossi, V. (2019). Interactions among fungicides applied at different timings for the control of Botrytis bunch rot in grapevine. *Crop Protection*, *120*, 30–33. https://doi.org/10.1016/j.cropro.2019.02.009
34. González-Rodríguez, R. M., Cancho-Grande, B., Torrado-Agrasar, A., Simal-Gándara, J., & Mazaira-Pérez, J. (2009). Evolution of tebuconazole residues through the winemaking process of Mencía grapes. *Food chemistry*, *117*(3), 529–537. https://doi.org/10.1016/j.foodchem.2009.04.030Get
35. Grosicka-Maciąg, E. (2011). Biological consequences of oxidative stress induced by pesticides. *Postepy Higieny i Medycyny Doswiadczalnej (Online)*, *65*, 357–366. https://doi.org/10.5604/17322693.948816
36. Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.
37. Hamidi-Esfahani, Z., Hejazi, P., Shojaosadati, S. A., Hoogschagen, M., Vasheghani-Farahani, E., & Rinzema, A. (2007). A two-phase kinetic model for fungal growth in solid-state cultivation. *Biochemical Engineering Journal*, *36*(2), 100–107. https://doi.org/10.1016/j.bej.2007.02.005
38. Hammer, Ø., Harper, D. A., & Ryan, P. D. (2001). *PAST*, *4.01*, Palaeontologia electronica. Retrieved September 5, 2022, from https://paleo.carleton.ca/2001_1/past/past.pdf
39. Hayes, B. M., Anderson, M. A., Traven, A., van der Weerden, N. L., & Bleackley, M. R. (2014). Activation of stress signalling pathways enhances tolerance of fungi to chemical fungicides and antifungal proteins. *Cellular and molecular life sciences*, *71*(14), 2651–2666. https://doi.org/10.1007/s00018-014-1573-8
40. Heipieper, H. J., Weber, F. J., Sikkema, J., Keweloh, H., & de Bont, J. A. (1994). Mechanisms of resistance of whole cells to toxic organic solvents. *Trends in Biotechnology*, *12*(10), 409–415. https://doi.org/10.1016/0167-7799(94)90029-9
41. Hou X, Xu Z, Zhao Y, Liu D. (2020). Rapid analysis and residue evaluation of six fungicides in grape wine-making and drying. *Journal of Food Composition and Analysis*, *89*, 103465. https://doi.org/10.1016/j.jfca.2020.103465
42. Hu, K., Zhao, H., Kang, X., Ge, X., Zheng, M., Hu, Z., & Tao, Y. (2022). Fruity aroma modifications in Merlot wines during simultaneous alcoholic and malolactic fermentations through mixed culture of *S. cerevisiae*, *P. fermentans*, and *L. brevis*. *LWT*, *154*, 112711. https://doi.org/10.1016/j.lwt.2021.112711
43. IARC (International Agency for Research on Cancer). (1993). *Agents Classified by the IARC Monographs, Volumes 1-119*. List of Classifications. Ochratoxin A, CAS No. 303-47-9. Retrieved September 5, 2022, from https://monographs.iarc.who.int/list-of-classifications
44. IARC (International Agency for Research on Cancer). (2018). *Agents Classified by the IARC Monographs, Volumes 1–119*. List of Classifications. 2,4-dichlorophenoxyacetic acid, CAS. No 94-75-7. Retrieved September 5, 2022, from https://monographs.iarc.who.int/list-of-classifications
45. Junior, W. J. L., Binati, R. L., Bersani, N., & Torriani, S. (2021). Investigating the glutathione accumulation by non-conventional wine yeasts in optimized growth conditions and multi-starter fermentations. *LWT*, *142*, 110990. https://doi.org/10.1016/j.lwt.2021.110990
46. Lash, L. H. (2005). Role of glutathione transport processes in kidney function. *Toxicology and applied pharmacology*, *204*(3), 329–342. https://doi.org/10.1016/j.taap.2004.10.004

47. Lu, S. C. (2013). Glutathione synthesis. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(5), 3143–3153. https://doi.org/10.1016/j.bbagen.2012.09.008

48. Kersh, D. M. E., Hammad, G., Donia, M. S., & Farag, M. A. (2022). A Comprehensive Review on Grape Juice Beverage in Context to Its Processing and Composition with Future Perspectives to Maximize Its Value. *Food and Bioprocess Technology*, 1–23. https://doi.org/10.1007/s11947-022-02858-5

49. Klarić, D. A., Klarić, I., Mornar, A., & Nigović, B. (2015). Evaluation of volatile compound and food additive contents in blackberry wine. *Food control*, *50*, 714–721. https://doi.org/10.1016/j.foodcont.2014.10.013

50. Kłosowski, G., Mikulski, D., Grajewski, J., & Błajet-Kosicka, A. (2010). The influence of raw material contamination with mycotoxins on alcoholic fermentation indicators. *Bioresource Technology*, *101*(9), 3147–3152. https://doi.org/10.1016/j.biortech.2009.12.040

51. Kochman, J., Jakubczyk, K., & Janda, K. (2021). Mycotoxins in red wine: Occurrence and risk assessment. *Food Control*, *129*, 108229. https://doi.org/10.1016/j.foodcont.2021.108229

52. Li, J. M., Si, H. Y., Yu, Y., Duan, H., Liang, D. M., Jiang, W. G., & Li, X. H. (2012). Effects of the residues of agricultural chemicals in grapes on winemaking. *Scientia Agricultura Sinica*, *45*(4), 743–751. https://doi.org/10.3864/j.issn.0578-1752.2012.04.015

53. Miraglia, M., & Brera, C. (2002). *Assessment of dietary intake of ochratoxin A by the population of EU member states–Report of experts participating in Task 3.2.7*. Directorate-General Health and Consumer Protection of the European Commission. Retrieved September 5, 2022, from https://food.ec.europa.eu/system/files/2016-10/cs_contaminants_catalogue_ochratoxin_task_3-2-7_en.pdf

54. Margalef-Català, M., Araque, I., Bordons, A., & Reguant, C. (2017). Genetic and transcriptional study of glutathione metabolism in *Oenococcus oeni*. *International Journal of Food Microbiology*, *242*, 61–69. https://doi.org/10.1016/j.ijfoodmicro.2016.11.013

55. Meca, G., Blaiotta, G., & Ritieni, A. (2010). Reduction of ochratoxin A during the fermentation of Italian red wine Moscato. *Food Control*, *21*(4), 579–583. https://doi.org/10.1016/j.foodcont.2009.08.008

56. Medina, Á., Mateo, R., Valle-Algarra, F. M., Mateo, E. M., & Jiménez, M. (2007). Effect of carbendazim and physicochemical factors on the growth and ochratoxin A production of *Aspergillus carbonarius* isolated from grapes. *International Journal of Food Microbiology*. *119*(3), 230–235. https://doi.org/10.1016/j.ijfoodmicro.2007.07.053

57. Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

58. Owens, C. W. I., & Belcher, R. V. (1965). A colorimetric micro-method for the determination of glutathione. *Biochemical Journal*, *94*(3), 705. https://doi.org/10.1042/bj0940705

59. Owsiak, A., Marchel, M., & Zyracka, E. (2021). Impact of pesticides used in the culture of the vine on the viability of the yeast *Saccharomyces cerevisiae* wine in chronological aging. *Journal of Microbiology, Biotechnology and Food Sciences*, *2021*, 48–51. https://doi.org/10.15414/jmbfs.2015.4.special1.48-51

60. Payá, P., Anastassiades, M., Mack, D., Sigalova, I., Tasdelen, B., Oliva, J., & Barba, A. (2007). Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multiresidue method in combination with gas and liquid chromatography and tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry*, *389*(6), 1697–1714. https://doi.org/10.1007/s00216-007-1610-7

61. Ritcharoon, B., Sallabhan, R., Toewiwat, N., Mongkolsuk, S., & Loprasert, S. (2020). Detection of 2, 4-dichlorophenoxyacetic acid herbicide using a FGE-sulfatase based whole-cell Agrobacterium biosensor. *Journal of Microbiological Methods*, *175*, 105997. https://doi.org/10.1016/j.mimet.2020.105997
62. Rollini, M., & Manzoni, M. (2006). Influence of different fermentation parameters on glutathione volumetric productivity by *Saccharomyces cerevisiae*. *Process Biochemistry*, *41*(7), 1501–1505. https://doi.org/10.1016/j.procbio.2006.02.011
63. Romanazzi, G, & Feliziani, E. (2014). *Botrytis cinerea* (Gray Mold). In Bautista-Baños S (Ed) *Postharvest decay*, *Control Strategies* (pp 131–146), Academic Press.
64. Rose, G., Lane, S., & Jordan, R. (2009). The fate of fungicide and insecticide residues in Australian wine grape by-products following field application. *Food chemistry*, *117*(4), 634–640. https://doi.org/10.1016/j.foodchem.2009.04.061
65. Rossouw, G. C., Holzapfel, B. P., Rogiers, S. Y., Gouot. J. C., & Schmidtke, L. M. (2019). Repercussions of four herbicides on reproductive and vegetative development in potted grapevines. *Australian Journal of Grape and Wine Research*, *25*(3), 316–326. https://doi.org/10.1111/ajgw.12395
66. Sala, C., Fort, F., Busto, O., Zamora, F., Arola, L., & Guasch, J. (1996). Fate of some common pesticides during vinification process. *Journal of agricultural and food chemistry*, *44*(11), 3668–3671. https://doi.org/10.1021/jf960218y
67. Samphao, A., Butmee, P., Saejueng, P., Pukahuta, C., Švorc, Ľ., & Kalcher, K. (2018). Monitoring of glucose and ethanol during wine fermentation by bienzymatic biosensor. *Journal of electroanalytical chemistry*, *816*, 179–188. https://doi.org/10.1016/j.jelechem.2018.03.052
68. Shen, F., Wu, W., Han, X., Wang, J., Li, Y., & Liu, D. (2021). Study on the occurrence law and green control of grape gray mold from the perspective of ecological balance. *Bioengineered*, *12*(1), 779–790. https://doi.org/10.1080/21655979.2021.1888578
69. Strober, W. (2015). Trypan blue exclusion test of cell viability. *Current protocols in immunology*, *111*(1), A3-B. https://doi.org/10.1002/0471142735.ima03bs111
70. Scariot, F. J., Delamare, A. P. L., & Echeverrigaray, S. (2022). The effect of chlorothalonil on *Saccharomyces cerevisiae* under alcoholic fermentation. *Pesticide Biochemistry and Physiology*, *182*, 105032. https://doi.org/10.1016/j.pestbp.2021.105032
71. Teixeira, M. C., Telo, J. P., Duarte, N. F., & Sá-Correia, I. (2004). The herbicide 2, 4-dichlorophenoxyacetic acid induces the generation of free-radicals and associated oxidative stress responses in yeast. *Biochemical and biophysical research communications*, *324*(3), 1101–1107. https://doi.org/10.1016/j.bbrc.2004.09.158
72. Torović, L., Lakatoš, I., Majkić, T., & Beara, I. (2020). Risk to public health related to the presence of ochratoxin A in wines from Fruška Gora. *LWT*, *129*, 109537. https://doi.org/10.1016/j.lwt.2020.109537
73. Viegas, C. A., Cabral, M. G., Teixeira, M. C., Neumann, G., Heipieper, H. J., & Sá-Correia, I. (2005). Yeast adaptation to 2,4-dichlorophenoxyacetic acid involves increased membrane fatty acid saturation degree and decreased OLE1 transcription. *Biochemical and biophysical research communications*, *330*(1), 271–278. https://doi.org/10.1016/j.bbrc.2005.02.158
74. Wen, S., Zhang, T., & Tan, T. (2005). Optimization of the amino acid composition in glutathione fermentation. *Process Biochemistry*, *40*(11), 3474–3479. https://doi.org/10.1016/j.procbio.2005.02.027
75. Yousefi, M., Khorshidian, N., & Mortazavian, A. M. (2021). Detoxification properties of microorganisms in foods. In *Microbial Biotechnology in Food and Health* (pp. 81–112). Academic Press.

76. Zhang, B., Liu, H., Xue, J., Tang, C., Duan, C., & Yan, G. (2022). Use of *Torulaspora delbrueckii* and *Hanseniaspora vineae* co-fermentation with *Saccharomyces cerevisiae* to improve aroma profiles and safety quality of Petit Manseng wines. *LWT*, 113360. https://doi.org/10.1016/j.lwt.2022.113360
77. Zhang, T., Wen, S., & Tan, T. (2007). Optimization of the medium for glutathione production in *Saccharomyces cerevisiae*. *Process biochemistry*, *42*(3), 454–458. https://doi.org/10.1016/j.procbio.2006.09.003

Tables

Table 1 – Biochemical characterization of cultures with *Saccharomyces cerevisiae* in synthetic must (YPD) with and without 2,4-D, procymidone and OTA

Analytical Parameters	Time (h)	Control treatment	Treatment 1	Treatment 2	Treatment 3
Biomass concentration (mg mL^{-1})	0	0.03 (±0) eA	0.02 (±0) eA	0.02 (±0) fA	0.03 (±0) fA
	24	0.12 (±0) dB	0.13 (±0) dA	0.09 (±0) eC	0.10 (±0) eC
	48	0.24 (±0) cA	0.26 (±0) cA	0.17 (±0) dB	0.14 (±0) dC
	72	1.15 (±0) aA	1.10 (±0.04) aAB	1.10 (±0) aAB	1.02 (±0) aB
	96	0.83 (±0) bB	0.84 (±0.02) bB	0.97 (±0.01) bA	0.94 (±0) bA
	168	0.86 (±0.02) bA	0.91 (±0.01) bA	0.65 (±0) cB	0.67 (±0.02) cB
Cell viability (%)	0	100 (±0) aA	100 (±0) aA	100 (±0) aA	100 (±0) aA
	24	96.87 (±0.05) fA	93.8 (±0.01) cB	92.82 (±0.29) fC	92.58 (±0.15) dC
	48	99.37 (±0) bA	94.20 (±0.04) cD	98.37 (±0) bB	97.11 (±0.01) bC
	72	98.93 (±0) cA	97.17 (±0.03) bB	95.8 (±0.01) cC	93.13 (±0.03) cD
	96	98.11 (±0.01) dA	92.29 (±0.19) dC	94.9 (±0.09) dB	91.83 (±0.09) eC
	168	97.82 (±0) eA	84.97 (±0.54) eD	93.6 (±0.15) eB	91.16 (±0.14) fC
Glutathione (mg L^{-1})	0	9.87 (±1.5) fB	10.0 (±0.77) eA	10.02 (±0.03) eA	9.19 (±0.06) fC
	24	11.45 (±0.63) eA	9.14 (±1.67) fD	9.46 (±0.34) eC	9.85 (±0.96) eB
	48	22.67 (±0.07) bC	15.74 (±1.45) dD	23.29 (±0.14) bB	29.92 (±0.13) aA
	72	22.52 (±0.50) cD	24.05 (±1.72) aC	26.2 (±0.74) aB	28.49 (±0.78) bA
	96	32.02 (±0.12) aA	16.6 (±2.33) cD	20.88 (±1.53) cC	28.38 (±0.24) cB
	168	19.88 (±0.06) dB	16.96 (±1.95) bA	18.41 (±0.37) dB	10.87 (±0.22) dC
Peroxidase (U mL^{-1})	0	1.45 (±0) fA	1.23 (±0.01) fB	1.17 (±0) fC	1.17 (±0) fC
	24	4.01 (±0.17) eA	3.80 (±0.01) eB	3.22 (±0.06) eC	3.22 (±0.11) eC
	48	7.67 (±0.01) cB	7.37 (±0.34) dC	10.55 (±0.09) dA	10.55 (±0.3) dA
	72	9.62 (±0.02) aB		11.33 (±0.01) bA	11.33 (±0.01) bA
	96				

	168	7.54 (±0.07) dC 8.17 (±0.02) bC	8.36 (±0.49) cC 9.04 (±0.13) aB 8.52 (±0.04) bB	10.69 (±0.05) cA 11.47 (±0.01) aA	10.69 (±0.06) cA 11.47 (±0.05) aA
Reducing sugar (mg mL^{-1})	0 24 48 72 96 168	202.0 (±0.26) aA 135.6 (±0.23) bB 9.7 (±0.01) cA 4.8 (±0.01) cdA 2.6 (±0) dAB 1.7 (±0.01) dB	202.7 (±0.48) aA 145.4 (±0.14) bA 10.1 (±0) cA 4.6 (±0) cA 2.73 (±0.01) cA 1.8 (±0) cB	200.5 (±0.27) aA 123.0 (±0.07) bC 7.2 (±0) cB 2.8 (±0.01) cB 2.43 (±0) cAB 2.3 (±0) cA	200.1 (±0.06) aA 121.3 (±0.04) bC 6.8 (±0.01) cB 3.2 (±0.04) dB 2.4 (±0) dB 2.1 (±0) dA
Ethanol (%)	0 24 48 72 96 168	0.05 (±0) cA 2.3 (±0.02) cA 8.8 (±0.16) bA 13.0 (±0.08) aA 14.4 (±0.78) aA 14.4 (±0.91) aA	0.05 (±0) cA 1.4 (±0.03) cB 7.6 (±0.25) bA 12.9 (±0.58) aA 14.7 (±0.2) aA 13.4 (±0.67) aA	0.04 (±0) cB 0.97 (±0.01) cC 8.13 (±0.50) bA 10.6 (±0.37) aB 10.8 (±0.46) aB 10.4 (±0.09) aB	0.04 (±0) cB 0.7 (±0) cD 8.9 (±0.33) aA 9.0 (±0.58) aB 7.4 (±0.48) abC 7.3 (±0.01) bC

Treatment 1: 6.73 mg L^{-1} 2,4-D + 2.24 mg L^{-1} procymidone. Treatment 2: 0.83 µg L^{-1} OTA. Treatment 3: 2.66 µg L^{-1} OTA. All experiments exhibited coefficient of variations below 5%. Different lowercase letters in a column show significant differences among days. Different uppercase letters on lines show significant differences among treatments ($p < 0.05$).

Table 2 – Maximum growth rate (μ_{max}) of cultures conducted in synthetic must (YPD) with and without 2,4-D, procymidone and OTA

Treatments	μ_{max} (h^{-1})	Exponential phase interval (h)	Reduction of μ_{max} (%)
Control treatment	0.0573	0-72	-
Treatment 1	0.0567	0-72	1
Treatment 2	0.0552	0-72	9
Treatment 3	0.0501	0-72	12.5

Treatment 1: 6.73 mg L^{-1} 2,4-D + 2.24 mg L^{-1} procymidone. Treatment 2: 0.83 µg L^{-1} OTA. Treatment 3: 2.66 µg L^{-1} OTA.

Table 3 – Redox-specific degradation and velocity of degradation in relation to GSH and PO of contaminants in alcoholic fermentation

Treatments	Tempo (h)	Redox-specific degradation (%deg/$_{GSH}$*U_{PO}*h)	Velocity of degradation in relation to GSH and PO (µg/GSH*U*h)
T1 2,4-D	0	-	-
	24	5.99	0.360
	48	1.61	0.072
	72	1.10	0.007
	96	1.18	0.028
	168	0.906	0.016
T1 Procymidone	0	-	-
	24	61.18	1.320
	48	10.23	0.224
	72	4.07	0.088
	96	4.3	0.094
	168	2.67	0.058
T2 OTA	0	-	-
	24	46.5	$3.83x10^{-7}$
	48	3.13	$2.63x10^{-8}$
	72	2.05	$1.78x10^{-8}$
	96	2.19	$1.96x10^{-8}$
	168	1.46	$1.27x10^{-8}$
T3 OTA	0	-	-
	24	38.09	$7.88x10^{-7}$
	48	2.17	$4.62x10^{-8}$
	72	1.54	$3.44x10^{-8}$
	96	1.57	$3.09x10^{-8}$
	168	2.76	$5.72x10^{-8}$

Treatment 1 (T1): 6.73 mg L^{-1} 2,4-D + 2.24 mg L^{-1} procymidone. Treatment 2 (T2): 0.83 µg L^{-1} OTA. Treatment 3 (T3): 2.66 µg L^{-1} OTA.

Figures

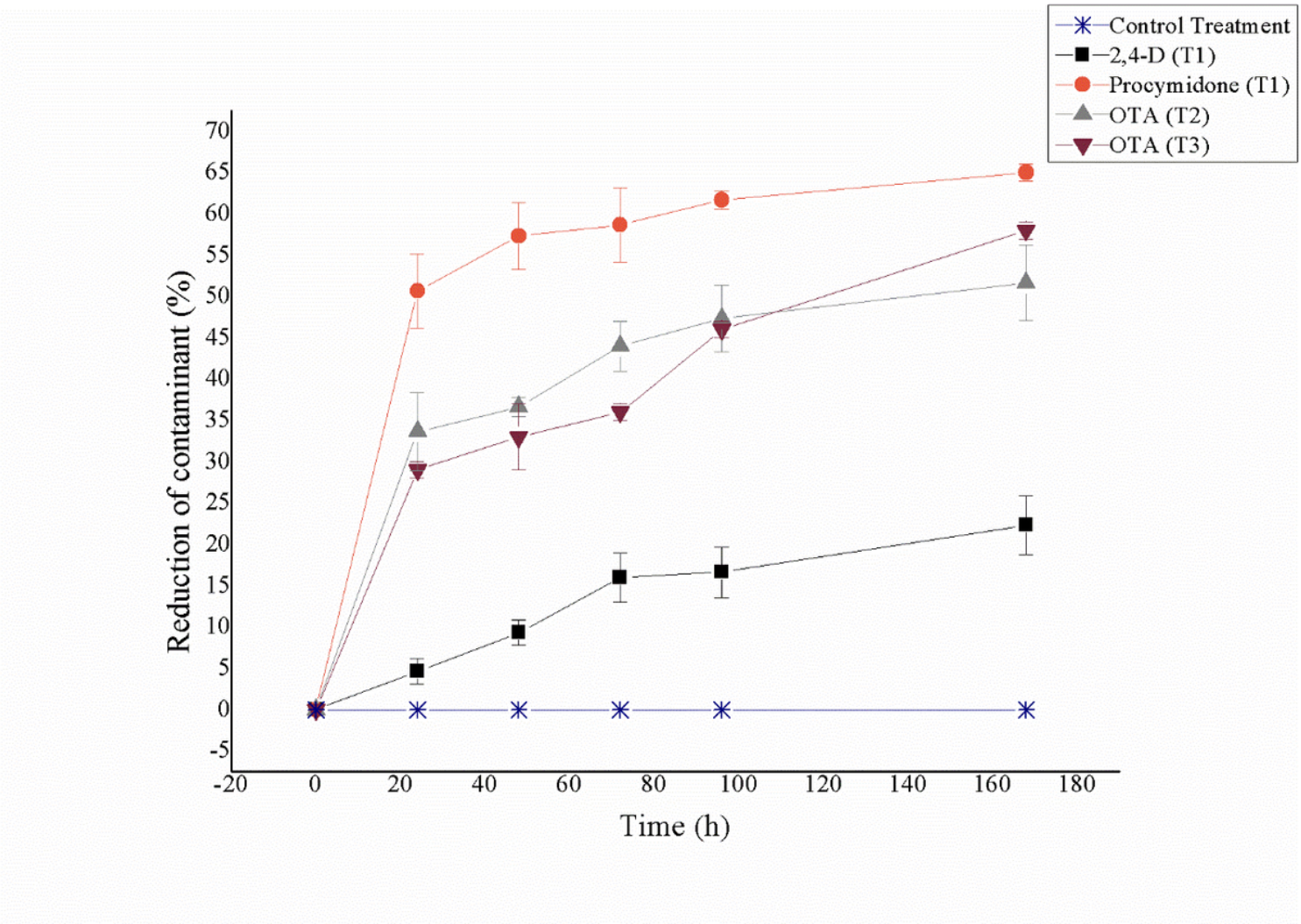

Figure 1

Reduction of 2,4-D, procymidone and OTA in alcoholic fermentation

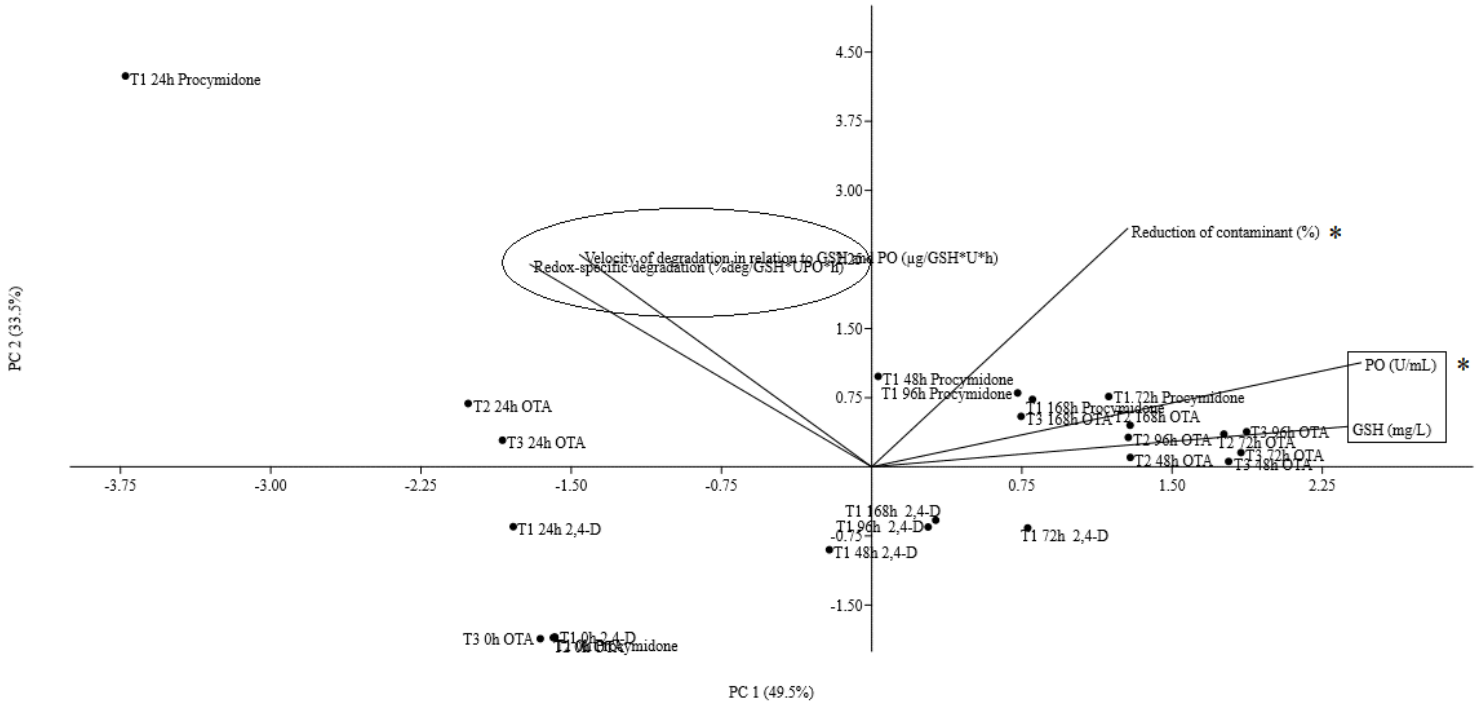

Figure 2

Principal Component Analysis of variables RSD, VDEGSH*PO, GSH, PO and reduction of contaminant