CIÊNCIA E TÉCNICA VITIVINÍCOLA
Ciência Téc. Vitiv. 38(1) 10-20. 2023

Article

MONITORING OF MYCOTOXINS AND PESTICIDES IN WINEMAKING

MONITORAMENTO DE MICOTOXINAS E PESTICIDAS NA VINIFICAÇÃO

Rafaela Xavier Giacomini[1], Maristela Barnes Rodrigues Cerqueira[1], Ednei Gilberto Primel[1], Jaqueline Garda-Buffon[1,*]

[1]Chemistry and Food School, Federal University of Rio Grande, Avenida Itália km 8, Campus Carreiros, Zip Code 96203-900, Rio Grande/Rio Grande do Sul, Brazil.

* Corresponding author: Tel.: + 55 53 32336796; e-mail: jaquelinebuffon@furg.br

(Received 12.09.2022. Accepted 12.01.2023)

SUMMARY

This study monitored concentrations of both pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and procymidone, and mycotoxin ochratoxin A (OTA) in stages of the winemaking process. Sampling was carried out in the usual vinification process of red wine in a winery between the steps to obtain must and alcoholic fermentation. The highest transference of contaminants in the process occurred in the crushing step to 2,4-D (100%) and maceration to OTA and procymidone (100%). Removal of contaminants in the winemaking process corresponded to 100%, with a half-life ($T_{1/2}$) longer for procymidone (216.5 h) and shorter for 2,4-D (38.5 h) and OTA (96 h). The processing factors (PFs) (0) for the contaminants, together with the data obtained, characterize winemaking as a process of reducing mycotoxin and pesticides. Results highlight the importance of fermentation to reduce contaminants and that yeasts promote detoxification

RESUMO

Este estudo monitorou, nas etapas do processo de vinificação, a transferência e a redução dos pesticidas 2,4-D, procimidona e da micotoxina ocratoxina A (OTA). A amostragem foi realizada durante processo usual de vinificação em vinho tinto em vinícola para o acompanhamento das concentrações de OTA e agrotóxicos entre as etapas de obtenção do mosto e fermentação alcoólica. A maior transferência dos contaminantes no processo ocorreu nas etapas de esmagamento para 2,4-D (100%) e maceração para OTA e procimidona (100%). A remoção dos contaminantes durante o processo de vinificação correspondeu a 100%, tendo tempo de meia vida ($T_{1/2}$) maior para procimidona (216,5 h) e menor para 2,4-D (38,5 h) e OTA (96 h). Os fatores de processamento (PFs) (0) para os contaminantes, juntamente com os dados obtidos caracterizam a vinificação como processo de redução de micotoxina e agrotóxicos. Os resultados destacam a importância da fermentação na redução de contaminantes, indicando que as leveduras promovem a detoxificação

Keywords: Fungicides, herbicides, ochratoxin A, *Saccharomyces cerevisiae*, wines.
Palavras-chave: Fungicidas, herbicidas, ocratoxina A, *Saccharomyces cerevisiae*, vinhos.

INTRODUCTION

The world's wine production was estimated to 260 million hectoliters (hL) in 2020. Brazil produced 3.6 million hL (OIV, 2021), mainly in Rio Grande do Sul (RS) state, which accounts for 62.51% of the national production (Mello and Machado, 2021). Moderate wine consumption has shown beneficial health effects associated with phenolic compounds, such as anthocyanins, quercetin and resveratrol (Giovinazzo and Grieco, 2015; Gabrielyan and Kazumyan *et al.*, 2018; Freire *et al.*, 2020; Ulrih *et al.*, 2020; Tıraş *et al.*, 2022). Despite its benefits, some contaminants, such as pesticides and mycotoxins, can be detected in this beverage sold worldwide and may cause negative effects on human health (Čepo *et al.*, 2018; Freire *et al.*, 2020; Čuš *et al.*, 2021).

The occurrence of pesticides is related to their use in vineyards to prevent fungal colonization. In addition, cross-contamination by pesticides from other agricultural crops (e.g., rice and soybeans), such as 2,4-dichlorophenoxyacetic acid (2,4-D), can also cause negative impacts on the wine sector (IBRAVIN, 2019). Even though this auxin-type herbicide is commonly used for exterminating weeds in grain and cereal crops (Rossouw *et al.*, 2019), effects of its spray particles may be seen several kilometers away from the target, particularly under conditions of high temperature, low relative humidity and prevailing winds (Felsot *et al.*, 2010).

In addition to the toxic effect on humans, contaminants, such as fungicides in beverage production, may also delay alcoholic fermentation

(Briz-Cid *et al.*, 2018), and influence the composition of volatile compounds in wines (Oliva *et al.*, 2015). Among fungicides, procymidone has been used for fighting diseases that affect vineyards worldwide, as shown by its occurrence in wines described by Čuš *et al.* (2010a), Doulia *et al.* (2016, 2017) and Romanazzi and Feliziani (2014). Pesticides application in order to protect vineyards may also be linked to the mycotoxin biosynthesis often related to ochratoxin (A) (OTA) (Gil-Serna *et al.*, 2018; Costa *et al.*, 2019), mainly by *Aspergillus carbonarius*, the main fungus contaminant in vines Medina *et al.*, 2007; Magistà *et al.*, 2021). This mycotoxin is described as harmful for the fermentative capacity of yeasts during alcoholic fermentation (Esti *et al*., 2012), and causes changes in wine composition. Thus, it affects its flavor and color (Freire *et al.*, 2020).

Therefore, methods of pesticide and mycotoxin decontamination have become a unique remediation alternative and must be investigated in the wine production chain. Emphasis should be given to biological conditions, with the use of yeasts, such as *Saccharomyces cerevisiae*, and usual conditions which compose usual winemaking processes (Tempère *et al.*, 2018). The use of this yeast is essential to the production process, since studies have shown its potential to remove OTA and pesticides, i.e., rates above 90% of contamination observed in wine (Csutorás *et al.*, 2013; Pan *et al.*, 2018).

Therefore, this study aimed to monitor concentrations of both pesticides 2,4-D and procymidone and mycotoxin OTA in stages of the winemaking process. Besides, this study, which was carried out on an industrial scale, contributed to the characterization of the winemaking as a process to reduce contamination of this widely consumed beverage.

MATERIALS AND METHODS

Pesticides and OTA

Both pesticides 2,4-D and procymidone and mycotoxin OTA with purity above 98% were purchased from Sigma-Aldrich Brazil. Stock solutions of every pesticide (1000 µg/mL) were prepared in acetonitrile (MeCN). OTA stock solution was prepared by dissolving 1 mg of mycotoxin in benzene: MeCN (98:2 v/v) to a concentration of 100 µg/mL (AOAC, 1995).

Sampling

On an industrial scale (1,200 L), sampling was carried out in the usual vinification process of red wine produced by a winery. Initially, grapes were stemmed. Grains were slightly broken and transported to oak barrels with the addition of pectolytic enzyme (3 g/hL) and sulfur dioxide (10 g/hL). Maceration and alcoholic fermentation took place in oak barrels under temperature control (22 °C) with inoculation of non-*Saccharomyces* yeast *Torulaspora delbrueckii* (3 g hL-1), along with *S. cerevisiae* (10 g/hL) (Zymaflore, Laffort, Bordeaux, France). 48 h after the beginning of alcoholic fermentation, 1 g/hL of *Oenococcus oeni* lactic acid bacteria was added to promote malolactic fermentation. Devatting (separation of solid and liquid parts) was carried out one week after the beginning of malolactic fermentation, which was completed within one week after devatting while alcoholic fermentation ended three weeks after the end of malolactic fermentation.

Samples were collected at the end of every stage of the process, corresponding to crushing of grapes to obtain must (0 h time), pre-fermentative maceration with addition of non-*Saccharomyces* yeast (24 h), addition of *S. cerevisiae* yeast (120 h), devatting (480 h), stop of alcoholic fermentation (1062 h), and end of alcoholic fermentation (1230 h). Samples were placed in 250 mL vials and kept frozen until analysis.

Determination of 2,4-D and procymidone

Extraction of herbicide 2,4-D and fungicide procymidone from must and wine samples in stages of the winemaking process were carried out by the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method described by Payá *et al.* (2007) with modifications, that is, elimination of sesquihydrated and dehydrated citrate salts. Extraction was performed with the use of MeCN while the extract was cleaned with magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) salts. The resulting extract was used for identifying and quantifying pesticides by liquid chromatograph with a diode array detector (LC–DAD) and gas chromatograph coupled to a mass spectrometer (GC–MS).

A chromatographic column Supelco® - Kromasil C18 (150 mm x 4.6 mm, 5 µm) was used in a Shimadzu Liquid Chromatograph (Kyoto, Japan) with a diode array detector (LC–DAD) to separate the herbicide 2,4-D. The mobile phase consisted of MeCN and acidified Mili-Q water (aqueous phosphoric acid solution 1:1 v/v; pH 3.0) 52:48 (v/v), as proposed by Caldas *et al.* (2009), with modifications, at flow rate of 0.8 mL/min, temperature of 25 ºC and retention time of 5.52 min. The wavelength used for identifying 2,4-D was 220.3 nm, as described by Caldas *et al*. (2009).

An RTX-5MS column (30 m x 0.25 mm ID, 0.25 µm) was used in a Shimadzu Gas Chromatograph (Kyoto, Japan) QP2010 Plus equipped with an automatic sampler (AOC-20i) coupled to a spectrometer mass with quadrupole mass filter (GC–MS) to separate the fungicide procymidone. The source was electron ionization (EI) with energy of 70 eV. Helium was

used as the carrier gas and the injection volume was 1 µL, as described by Barbosa *et al*. (2020).

Determination of OTA

OTA extraction from samples was carried out by the QuEChERS method described by Fernandes *et al*. (2013). Extraction was performed with acidified MeCN (1% acetic acid) while partitioning was done with the addition of a mixture of salts containing $MgSO_4$, NaCl, tribasic sodium citrate dihydrate and sodium citrate dibasic sesquihydrate. The supernatant (1.5 mL) was transferred to an amber flask and the solvent was evaporated under a stream of nitrogen. The dry extract was resuspended in 1 mL of mobile phase (60% MeCN, 40% Mili-Q water acidified with 1% acetic acid), followed by chromatographic analysis.

In separation, identification and quantification, the analytical chromatographic column Supelco® - Kromasil C18 (5 µm and 150 mm x 4.6 mm) was used, kept 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 was composed of MeCN: 1% acidified water with acetic acid (60:40, v/v), and flow rate was 0.8 mL/min, as described by Garcia *et al.* (2020).

Method Validation of 2,4-D, procymidone and OTA

Methods of 2,4-D, procymidone and OTA determination were validated according to the guidelines issued by the European Commission – EC (EC, 2006; 2019) and the National Health Surveillance Agency – ANVISA (ANVISA, 2017). Concentrations used for constructing the analytical curves in the matrix extract (must and wine) ranged from 0.05 to 2.5 µg/mL, resulting in seven levels of 2,4-D; from 0.0075 to 1.5 µg/mL, totaling twelve levels of procymidone; and from 0.1 to 3.0 ng/mL, totaling eleven levels of OTA. Based on analytical curves, linearity of the methods was evaluated, considering the coefficient of determination (R^2). Recovery was determined by fortification in both matrices (must and wine) at three concentrations: 0.07, 0.5 and 1.0 µg/mL for 2,4-D; 0.075, 0.5 and 1.0 µg/mL for procymidone; 0.13, 1.0 and 3.0 ng/mL in must and 0.13, 1.0 and 2.5 ng/mL in wine for OTA.

Data treatment

Dissipation of contaminants in the winemaking process was estimated by the first-order kinetics equation and calculated by Equation 1, where C (µg/L for mycotoxin and mg/L for pesticides) is the residual amount of the contaminant at time t, C0 is the initial residual amount of the contaminant, and k (h-1) is the dissipation constant. Half-lives (T1/2) of every contaminant were calculated by Equation 2, (Pan *et al*., 2018; Hou *et al*., 2020).

$$C = C_0 e^{-kt} \quad \textbf{Eq. 1}$$

$$T_{1/2} = \frac{(\ln 2)}{k} \quad \textbf{Eq. 2}$$

Processing factors (PFs) were calculated according to the recommendations issued in the Joint Meeting on Pesticide Residues (JMPR, 2006) by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to show residue disposal in processed products - Equation 3. PFs values < 1 show that there is decrease in contaminant levels after raw material processing, while values > 1 mean that the contaminant level increased on processing (Pan et al., 2018; Hou et al., 2020).

$$PFs = \frac{\text{residuale level in processed product}}{\text{residuale level in commodity to be processed}} \quad \textbf{Eq.3}$$

The removal and transference of contaminants in the winemaking process were evaluated by Equations 4 and 5 (Hou *et al.*, 2020).

Removal (%) =

$$\left[1 - \frac{\text{residue of contaminant in the processed product}}{\text{contaminnat residue at the beginning of the process}}\right] * 100\% \quad \textbf{Eq. 4}$$

Transference (%) =

$$\left[\frac{\text{residue of contaminant in the processed product}}{\text{contaminnat residue at the beginning of the process}}\right] * 100\% \quad \textbf{Eq. 5}$$

PAST 2.04 software (Hammer *et al*., 2001) was used for the principal component analysis (PCA) of the data set (triplicate) on concentration, removal and transference of OTA, 2,4-D and procymidone.

RESULTS AND DISCUSSION

Validation of the method for 2,4-D, procymidone and OTA determination

In this study, quality parameters of determination of 2,4-D, procymidone and OTA exhibited results that showed the analytical quality of the proposed methods (Table I). Mean recoveries of herbicide 2,4-D ranged between 88 and 90% in must and from 72 to 100% in wine at three concentration levels (0.07, 0.5 and 1.0 µg/mL) with relative standard error (RSD) varying from 1.0 to 5.6% in must and from 1.4 to 8.5% in wine. Fungicide procymidone showed recoveries ranging from 99 to 104% in must and from 78 to 86% in wine with RSD from 1.5 to 4.9% in must and from 2.5 to 7.6% in wine. The proposed method of OTA mycotoxin showed recoveries between 98 to 112% in must (RSD between 2.5 and 2.6%) and from 104 to 108% in wine (RSD between

Table I

Analytical parameters of 2,4-D, procymidone and OTA quantification

Analytical parameters	2,4-D	Procymidone	OTA
Analytical curve (must)	y = 36720x – 1660.3	y = 20959x + 3739.4	y = 18927x – 1621
Linear range	0.070–2.5 µg/mL	0.075–1.5 µg/mL	0.13–3.0 ng/mL
Determination coefficient (R^2)	0.9997	0.9966	0.9985
Correlation coefficient (r)	0.9998	0.9982	0.9992
LOQ of the method	0.070 µg/mL	0.075 µg/mL	0.12 ng/mL
LOD of the method	0.050 µg/mL	0.023 µg/mL	0.038 ng/mL
Analytical curve (wine)	y = 35230x – 2026.9	y = 4732.6x – 1611.4	y = 15698x – 1272.3
Linear range	0.070–2.5 µg/mL	0.075–1.5 µg/mL	0.13–2.5 ng/mL
Determination coefficient (R^2)	0.9992	0.9855	0.9993
Correlation coefficient (r)	0.9995	0.9927	0.9996
LOQ of the method	0.070 µg/mL	0.075 µg/mL	0.12 ng/mL
LOD of the method	0.050 µg/mL	0.023 µg/mL	0.038 ng/mL

Limit of quantification (LOQ); Limit of detection (LOD); 2,4-dichlorophenoxyacetic acid (2,4-D); Ochratoxin A (OTA).

0.8 and 2.8%). These results show reliability to quantify concentrations of 2,4-D, procymidone and OTA (ANVISA, 2017; EC, 2006; 2019).

Levels of OTA and pesticides in the winemaking process

Both pesticides procymidone and 2,4-D and mycotoxin OTA were detected in steps of the winemaking process, as shown in Table II. However, for each contaminant there is a significant transference step. The highest levels of OTA and procymidone were detected in pre-fermentative maceration (contact of must with grape skin). In this step, OTA concentrated 2.0 times and procymidone 1.5 times. Some studies suggest that the highest transference of OTA and fungicides in the winemaking process occurs during maceration. This stage promotes extraction of anthocyanins from grape skin, thus, generating the desired color of red wines, but it also promotes extraction of other compounds, such as contaminants found in the matrix (González-Rodríguez *et al.*, 2009; Čepo *et al.*, 2018; Kochman *et al.*, 2021).

Regarding herbicide 2,4-D, the highest transference was observed in the crushing step (Table II), in which its concentration was 4-fold higher than in the other steps. In this step, grape skin breaks to facilitate maceration (Kochman *et al.*, 2021). This herbicide is not recommended for growing grapes since its contamination in vineyards is due to drift (Felsot *et al.*, 2010; Rossouw *et al.*, 2019). High transference of this herbicide may also be related to polarity. Besides, it has high solubility in water (24,300 mg/L) and low octanol-water partition coefficient (log Kow - 0.82) (Lewis *et al.*, 2006; FAO, 2020).

Some compounds tend to be more easily detected than others in liquid samples due to the wide range of physicochemical properties of pesticides (polarity, solubility in water, K_{ow}) (Pichon *et al.*, 1998). According to Manjarres-López *et al.* (2021), the more soluble pesticides are more easily detected in water; besides, the less soluble they are, the more easily detected in the soil. Therefore, more apolar and less water-soluble pesticides, such as procymidone (2.46 mg/L of water solubility and 3.3 log K_{ow}) (FAO, 2001; Lewis *et al.*, 2006), require the maceration step to be transferred from grapes to must in the winemaking process (Table II).

Removal of the three contaminants (OTA, 2,4-D and procymidone) was observed in alcoholic fermentation with the addition of *S. cerevisiae* yeast. In the fermentation process, evidence exists that yeasts may cause dissipation of OTA residues and pesticides by degradation (Angioni *et al.*, 2007; Čuš *et al.*, 2010b; Freire *et al.*, 2019; Schusterova *et al.*, 2021) and adsorption (Meca *et al.*, 2010; Petruzzi *et al.*, 2015), even when deposited as lees (Bejaoui *et al.*, 2006; Caboni and Cabras, 2010; Petruzzi *et al.*, 2015). According to Petruzzi *et al.* (2015), decontamination is linked to the winemaking process itself, mainly to fermentation, in which microorganisms generally recognized as safe (GRAS) are used.

They can activate their own mechanisms of detoxification and produce molecules that have

Table II

Levels of pesticides and OTA in stages of the winemaking process

Stages of the winemaking process	OTA (µg/L ([1]RSD %))	2,4-D (mg/L ([1]RSD %))	Procymidone (mg/L ([1]RSD %))
Crushing of grape grains	0.160 ± 5.86	27.80 ± 0.73	2.24 ± 2.16
Pre-fermentative maceration with addition of non-*Saccharomyces* yeast	0.830 ± 3.48	6.73 ± 8.90	9.09 ± 0.32
Addition of *S. cerevisiae* yeast	0.380 ± 9.13	3.21 ± 2.70	5.86 ± 6.28
Devatting	nd	nd	<LOQ
Stop in alcoholic fermentation	nd	nd	<LOQ
End of alcoholic fermentation	nd	nd	nd

nd - no contaminant detected; Limit of quantification (LOQ): 0.075; [1] Results expressed as means (n = 3) ± relative standard error (RSD); Ochratoxin A (OTA); 2,4-dichlorophenoxyacetic acid (2,4-D).

absorbing power in the cell structure (Petruzzi *et al.*, 2015; Kumar *et al.*, 2020; Boeira *et al.*, 2021).

The process of separating wine lees after alcoholic fermentation was studied by Čuš *et al.* (2010b), who reported the occurrence of procymidone in lees of 1.09 mg/kg, at initial concentration of 5.34 mg/kg. However, in the case of OTA, Petruzzi *et al.* (2014) showed that *S. cerevisiae* W13 yeast strain was able to remove about 57% of OTA at 30 °C after 72 h of incubation. Petruzzi *et al.* (2015) evaluated the ability of five strains of *S. cerevisiae* yeasts to remove OTA in red grape must on a laboratory scale. The authors observed that yeasts were able to reduce OTA levels by 20.34–76.44% after alcoholic fermentation, suggesting that OTA removal is affected by the type of yeast strain, temperature (25–30 ºC), sugar concentration (200–250 g/L) and addition of nitrogen supplied as diammonium phosphate (DAP) (300 mg/L). The highest percentage of OTA removal (76.44%) was observed when the strain was subjected to temperature of 30 ºC, an initial sugar concentration of 250g/L and addition of 300 mg/L DAP.

Farbo *et al.* (2016) showed significant adsorption (above 80%) of the OTA level in grape juice, which was achieved by immobilized yeast cells after 48 h of incubation. In addition, Abrunhosa *et al.* (2014) reported that *Pediococcus parvulus* bacterial strains degraded 90% of OTA after 19 h of incubation. Lactic acid bacteria and yeast are the most important microorganisms that may be used for decontamination of mycotoxins and pesticides (Bangar *et al.*, 2021; Yousefi *et al.*, 2021). In our study, the microorganisms (lactic acid bacteria and yeasts) were used in the winemaking process, which can also be characterized by the total elimination of the three contaminants at the end of the process.

Dissipation of OTA and pesticides in the winemaking process

Results of dissipation of the three contaminants in the winemaking process (Table III) showed that half-life of procymidone was 216.5 h longer than half-lives of other contaminants, 2,4-D and OTA ($T_{1/2}$= 38.5 and 96 h, respectively). Procymidone has low acute oral toxicity; oral LD_{50} in rats and mice has been reported in the literature as > 5000 mg/ g body weight (FAO, 2001; Rifai *et al.*, 2013). Acute oral toxicity of 2,4-D in rats is represented by an LD_{50} value of 699 mg/kg body weight (Munro *et al.*, 1992; Bus and Hammond, 2007; FAO, 2020). Oral LD_{50} values of OTA in rats and mice are 20–30 and 46–58 mg/kg of body weight, respectively (FAO/WHO, 2001).

Thus, OTA and 2,4-D are classified as possible carcinogens to humans (Group 2B) by the International Agency for Research on Cancer (IARC, 1993; 2018). Effectiveness of detoxification of different toxic compounds, such as pesticides and mycotoxins, due to the activity of yeasts, depends on several factors, such as the strain type, concentration, pH of the medium and incubation time in the process (Yousefi *et al.*, 2021). 2,4-D and OTA have high toxicity than procymidone and may be metabolized in shorter process time due to their effect on microorganisms in the fermentation medium (120 h or 5 days), as shown in Table III. There was removal of 12 and 54% to both contaminants between the steps of pre-fermentative maceration and addition of yeasts. In contrast, in the case of fungicide procymidone, removal was 35% between these steps, suggesting that low toxicity of the fungicide at high residual concentrations can be observed in other steps of the winemaking process (1062 h or 44 days) (Table III). These data show that *S. cerevisiae* yeast has medium detoxification power, which may be

associated with its metabolism or adsorption capacity, in addition to the contaminant toxicity (Petruzzi *et al.*, 2015).

Table III

Process time (T), first-order constant (k), half-life time (T½) and correlation coefficient (r) of dissipation of compounds in the winemaking process

Compound	Winemaking process			
	T (h^{-1})	k (h^{-1})	r	$T_{½}$ (h)
OTA (µg/L)	120.0	-0.007200	1.000	96.00
2,4-D (mg/L)	120.0	0.01800	1.000	38.50
Procymidone (mg/L)	1062.0	0.003200	1.000	216.50

Process time (T); First-order constant (k); Half-life time (T½); Correlation coefficient (r); 2,4-dichlorophenoxyacetic acid (2,4-D); Ochratoxin A (OTA).

Transference and removal of contaminants

Removal and transference of OTA, procymidone and 2,4-D and PFs that demonstrate the effect of the winemaking process on the residual levels of these contaminants are shown in Table IV. PFs values > 1 mean that the contaminant level increased on processing (Pan *et al.*, 2018; Hou *et al.*, 2020). Thus, results demonstrate that the highest transference occurred in the maceration step in the cases of OTA and procymidone, 100% (PFs = 1), that is, contaminants were concentrated in this step. However, the highest transference 100% (PFs = 1) of 2,4-D occurred at the beginning of the process (grape crushing) (Table IV).

Transference of contaminants from grapes to must and from must to wine depends on the winemaking process and on physicochemical properties of contaminants, mainly lipophilicity (log K_{ow}) and solubility (Alister *et al.*, 2014). As previously reported, pesticides with high water solubility and low lipophilicity, including herbicide 2,4-D (solubility in water 24,300 mg/L and log K_{ow} -0.82) (Lewis *et al.*, 2006; FAO, 2020), may be less soluble in the system, that is, constituents found in grape skins and seeds, such as lignin, cellulose, hemicellulose, sugar, pectin, protein, polyphenols, minerals and lipids (Jiang *et al.*, 2011; Gowman *et al.*, 2019; Yoon *et al.*, 2021), can adsorb them and contribute to the decrease (Yoon *et al.*, 2021) in the crushing step, differing from other contaminants that showed higher transference in the maceration step (Table IV).

Other results observed in the winemaking process, mainly in the devatting step are shown in Table IV. In this step, both pesticides procymidone and 2,4-D and mycotoxin OTA were removed between 99 and 100%, respectively, with PF values below 1 (0 for the three contaminants). These values suggest that the winemaking process reduces concentrations of contaminants in red wines.

Similar results were found by Pan *et al.* (2018) and Hou *et al.* (2020), who demonstrated on a laboratory scale that PFs of different pesticides (zoxamide, boscalid, picoxystrobin, fluopicolide, 2,6-dichlorobenzamide, pyraclostrobin and mandipropamid) were below 1 (factor of removal) after the winemaking process, thus, highlighting that the process may reduce pesticide residues in wines.

Results of great relevance were found in the case of different contaminants with 100% elimination at the end of alcoholic fermentation (Table IV). Regarding mycotoxin OTA, the highest transference (100%) was observed from grape to must in maceration, in comparison with the other contaminants under investigation (Table IV). Some studies reported that OTA contents detected in red wines are more frequent, mainly due to mandatory maceration of grape skin, which can enhance OTA extraction, as observed in the following ranges: from 0.02 to 0.134 µg/L (Torović *et al.*, 2020); 0.29 µg/L (Freire *et al.*, 2017); 0.45 µg/L (Remiro *et al.*, 2013); and 2.47–2.77 µg/L (Abreu *et al.*, 2016). In the present work, Table IV shows that there was neither detection of 2,4-D and OTA contaminants in the devatting step nor detection of procymidone at the end of the alcoholic fermentation (100% elimination).

For a wine to be considered dry, it must contain less than 4 g/L of sugar (expressed in grams of glucose per liter) (Brasil, 2014). When the yeasts stopped fermenting, the wine had 7.5 g/L of sugar. Stop in fermentation can harm ethanol production, generate costs with oenological inputs to carry out refermentation and lead to losses in organoleptic characteristics of wines (González-Álvarez *et al.*, 2012; Briz-Cid *et al.*, 2018).

Table IV

Removal, transference and PFs of contaminants in the winemaking process

Stages	Removal (%)			Transference (%)			PFs		
	OTA	2,4-D	Procy	OTA	2,4-D	Procy	OTA	2,4-D	Procy
Crushing of grape grains	*	0	*	*	**100	*	-	1.00	-
Pre-fermentative maceration	0	76.0	0	**100	nd	**100	1.00	0.24	1.00
Addition of *S. cerevisiae*	54.0	88.0	35.0	nd	nd	nd	0.45	0.11	0.64
Devatting	100.0	100.0	99.0	nd	nd	nd	0.00	0.00	0.00
Stop in fermentation	100.0	100.0	99.0	nd	nd	nd	0.00	0.00	0.00
End of fermentation	100.0	100.0	100.0	nd	nd	nd	0.00	0.00	0.00
Total	100.0	100.0	100.0	nd	nd	nd	0.00	0.00	0.00

*Increased concentration; **Higher concentration detected; PF_S: Processing factors; nd - no increase in concentration detected; 2,4-dichlorophenoxyacetic acid (2,4-D); Ochratoxin A (OTA); Procymidone (Procy).

Weak acids, such as 2,4-D, are known to have a negative impact on yeasts performance, thus, restricting efficiency in ethanol production and other products derived from their fermentative activity (Cabral *et al.*, 2003; Ndukwe *et al.*, 2020). Cabral *et al.* (2003) evaluated, on a laboratory scale, the inhibitory effect of 2,4-D on *S. cerevisiae* growth. The authors reported that this effect is strongly dependent on pH of the fermentation medium (from 2.5 to 6.5); the yeast strain (W303.1b) grown at 30 °C in MM2 growth medium exposed to increasing concentrations of 2,4-D was affected at pH 3.0, leading to a reduction in growth and loss of cell viability. Weak lipophilic acids, including 2,4-D, exert negative effects on yeasts when they diffuse into cells in their non-ionized state, because their pH is lower than the pKa of the yeast growth medium (Cabral *et al.*, 2003; Ndukwe *et al.*, 2020).

In the present study, alcoholic fermentation conducted by *S. cerevisiae* occurred at pH 3.1–3.2, close to the pKa (2.73) of 2.4-D (Cabral *et al.*, 2003) on an industrial scale. Therefore, stop in fermentation may also have been influenced by pH and toxicity of contaminants in the medium. It should be mentioned that herbicide 2,4-D is not recommended for growing vines. The study reported by this paper evaluated its total elimination (Table IV), highlighting the importance of reducing levels of contaminants in the winemaking process.

Briz-Cid *et al.* (2018) assessed the influence of fungicides on sensory properties and flavonoid composition of red wines. The authors observed that fermentation kinetics was influenced by fungicides. Wine grapes treated with boscalide (200 mg/mL) combined with kresoxim-methyl (100 mg/mL) exhibited initial delay in alcoholic fermentation. Furthermore, the authors described that residues of these fungicides may affect sensory quality of wine and decrease intensity of fruity aromas in wine by 42% and 59%, respectively.

The wide range of physicochemical (polarity, water solubility) and toxicological properties of the product is a consequence of the different levels of removal and transference of these contaminants in the winemaking process (Table IV). This study is extremely relevant because it enables to observe and describe the winemaking process related to the elimination/removal of contaminants so as to contribute to and strenghten the wine sector.

To better understand the relationship between removal of contaminants and stages of the winemaking process, the PCA was performed (Figure 1), highlighting three regions. The first component (PC 1) explains 68% of total variance. Therefore, almost 2/3 of information found in 9 database variables can be encompassed by this component. The second component (PC 2) explains 28% of total variance. Therefore, 96% of total variance is explained by only two components.

The circled region encompasses PFs of OTA, 2,4-D and procymidone. The Pearson's correlation shows positive and significant relation ($R = 0.99997$, $p = 1.7x10^{-5}$) between PFs of 2.4-D and PFs of OTA and ($R = 0.98542$, $p = 0.0003$) between PFs of procymidone and PFs of 2,4-D. It shows that, in grape processing, there is removal in contaminant levels (Table II), mainly related to *S. cerevisiae*

yeast, and points out that alcoholic fermentation reduces contamination of wines.

Another region framed by a square encloses the removal of OTA, procymidone and 2,4-D in the winemaking process, and shows that, from the devatting step onwards, removal of procymidone was 99% while removal of OTA and 2,4-D was 100%. At the end of the alcoholic fermentation, the three contaminants (100%) were completely removed (Tables II and IV). Based on these results, winemaking can be characterized as a process of reducing contaminants, mycotoxins and pesticides.

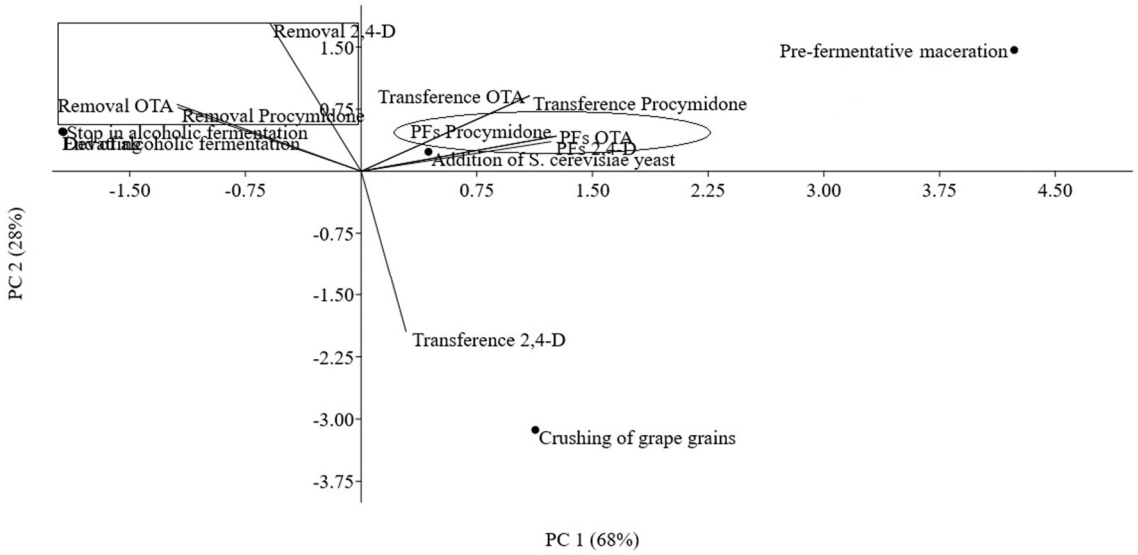

Figure 1. Principal Component Analysis of the following variables: Processing Factors, Removal and Transference of OTA, 2,4-D and procymidone.

CONCLUSIONS

The occurrence of three contaminants – mycotoxin OTA, fungicide procymidone and herbicide 2,4-D – was observed in the winemaking process. The highest transference of procymidone and OTA were detected in the maceration step (100%) and of 2,4-D, in the crushing step (100%). In the winemaking process, the highest dissipation was for procymidone ($T_{1/2}$ = 216.5 h). Removal of contaminants at the end of the process was 100% and PFs were below 1, thus, showing that the winemaking process can reduce their residues in wine. Results highlight the importance of fermentation to reduce contaminants and that yeasts promote detoxification.

ACKNOWLEDGEMENTS

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.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES

Abreu P.S., Terra M.F., Prado G., Santiago W.D., das Graças Cardoso M., Valeriano C., Batista L.R., 2016. Ochratoxin A in wines and evaluation of consumer exposure. *Food Public. Health.*, **6**, 107–114.

Abrunhosa L., Inês A., Rodrigues A.I., Guimarães A., Pereira V.L., Parpot P., Mendes-Faia A., Venâncio A., 2014. Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. *Int. J. Food Microbiol.*, **188**, 45–52.

Alister C., Araya M., Morandé J.E., Volosky C., Torrico J.S., Cordova A., Kogan M., 2014. Effects of wine grape cultivar, application conditions and the winemaking process on the dissipation of six pesticides. *Cienc. Investig. Agrar.*, **41**, 375–386.

Angioni A., Caboni P., Garau A., Farris A., Orro D., Budroni M., Cabras P., 2007. *In vitro* interaction between ochratoxin A and different strains of *Saccharomyces cerevisiae* and *Kloeckera apiculata*. *J. Agric. Food Chem.*, **55**, 2043–2048.

ANVISA, 2017. Resolução da diretoria colegiada - RDC nº 166, de 24 de julho de 2017 validação de métodos analíticos. Diário Oficial da União, 141. Available at: http://antigo.anvisa.gov.br/documents/10181/2721567/RDC_166_2017_COMP.pdf/d5fb92b3-6c6b-4130-8670-4e3263763401#:~:text=Objetivo-,Art.,objeto%20de%20an%C3%A1lise%20pela%20Anvisa (accessed on 02.09.2022).

AOAC, 1995. Official Methods of Analysis of International. (16th ed.). AOAC International, Arlington.

Bangar S.P., Sharma N., Kumar M., Ozogul F., Purewal S.S., Trif M., 2021. Recent developments in applications of lactic acid bacteria against mycotoxin production and fungal contamination. *Food Biosci.*, **44**, 101444.

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. *Rev. Mundi Eng. Tecn. Gest.*, **5**, 280–01, 280–16.

Bejaoui H., Mathieu F., Taillandier P., Lebrihi A., 2006. Biodegradation of ochratoxin A by *Aspergillus* section Nigri species isolated from French grapes: a potential means of ochratoxin A decontamination in grape juices and musts. *FEMS Microbiol. Lett.*, **255**, 203–208.

Brasil, 2014. Decreto nº 8.198, de 20 de fevereiro de 2014. Regulamenta a Lei no 7.678, de 8 de novembro de 1988, que dispõe sobre a produção, circulação e comercialização do vinho e derivados da uva e do vinho. Diário Oficial da República Federativa do Brasil. Available at: https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/decreto-no-8-198-de-20-de-fevereiro-de-2014.pdf (accessed on 02.09.2022).

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 Chem.*, **340**, 127935.

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. *J. Food Compos. Anal.*, **66**, 185–192.

Bus J.S., Hammond L.E., 2007. Regulatory progress, toxicology, and public concerns with 2,4-D: where do we stand after two decades? *Crop Prot.*, **26**, 266–269.

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**, 47–54.

Caboni P., Cabras P., 2010. Pesticides' influence on wine fermentation. *Adv. Food Nutr. Res.*, **59**, 43–62.

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. *J. Braz. Chem. Soc.*, **20**, 125–132.

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 Chem.*, **246**, 394–403.

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 Addit. Contam. - Chem. Anal. Control Expo. Risk Assess.*, **36**, 1860–1870.

Csutorás C., Rácz L., Rácz K., Főtő P., Forgó P., Kiss A., 2013. Monitoring of ochratoxin A during the fermentation of different wines by applying high toxin concentrations. *Microchem. J.*, **107**, 182–184.

Čuš F., Česnik H.B., Bolta Š.V., 2021. Pesticide residues, copper and biogenic amines in conventional and organic wines. *Food Control*, **132**, 108534.

Čuš F., Česnik H.B., Bolta Š.V., Gregorčič A., 2010a. Pesticide residues and microbiological quality of bottled wines. *Food Control*, **21**, 150–154.

Čuš F., Česnik H.B., Bolta Š.V., Gregorčič A., 2010b. Pesticide residues in grapes and during vinification process. *Food Control*, **21**, 1512–1518.

Doulia D.S., Anagnos E.K., Liapis K.S., Klimentzos D.A., 2016. Removal of pesticides from white and red wines by microfiltration. *J. Hazard. Mater.*, **317**, 135–146.

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.

EC, 2006. Commission Regulation No 401/2006 of 23 February 2006, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of European Communities L, 70/12.

EC, 2019. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. SANTE/12682/2019. Available at: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf (accessed on 02.09.2022).

Esti M., Benucci I., Liburdi K., Acciaro G., 2012. Monitoring of ochratoxin A fate during alcoholic fermentation of wine-must. *Food Control*, **27**, 53–56.

FAO, 2001. FAO specifications and evaluations for plant protection products: procymidone. Evaluation report: 383/2001. Available at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/procymid.pdf (accessed on 02.09.2022).

FAO, 2020. FAO specifications and evaluations for agricultural pesticides: 2,4-D (2,4-dichlorophenoxy)acetic acid. Evaluation report: 1/2020. Available at: http://www.fao.org/3/cb0999en/cb0999en.pdf (accessed on 02.09.2022).

FAO/WHO, 2001. Ochratoxin A. *In: Safety evaluation of certain mycotoxins in food*, *WHO food additives series 47*. 281–387. World Health Organisation: Switzerland.

Farbo M.G., Urgeghe P.P., Fiori S., Marceddu S., Jaoua S., Migheli Q., 2016. Adsorption of ochratoxin A from grape juice by yeast cells immobilised in calcium alginate beads. *Int. J. Food Microbiol.*, **217**, 29–34.

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 *J. Environ. Sci. Health - B Pestic. Food Agric. Contam. Wastes.*, **46**, 1–23.

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 Res. Int.*, **54**, 293–301.

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.

Freire L., Furtado M.M., Guerreiro T.M., Da Graça J.S., Da Silva B.S., Oliveira D. N., Catharino R.R., Sant'ana A.S., 2019. The presence of ochratoxin A does not influence *Saccharomyces cerevisiae* growth kinetics but leads to the formation of modified ochratoxins. *Food Chem. Toxicol.*, **133**, 110756.

Freire L., Passamani F.R.F., Thomas A.B., Nassur R.D.C.M.R., Silva L.M., Paschoal F.N., Pereira G.E., Prado G., Batista L.R., 2017. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. *Int. J. Food Microbiol.*, **241**, 181–190.

Gabrielyan A., Kazumyan K., 2018. The investigation of phenolic compounds and anthocyanins of wines made of the grape variety karmrahyut. *Ann. Agrar. Sci.*, **16**, 160–162.

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 Res. Int.*, **131**, 109039.

Gil-Serna J., Vázquez C., González-Jaén M., Patiño B., 2018. Wine contamination with ochratoxins: A Review. *Beverages*, **4**, 6.

Giovinazzo G., Grieco F., 2015. Functional properties of grape and wine polyphenols. *Plant Foods Hum. Nutr.*, **70**, 454–462.

González-Álvarez M., González-Barreiro C., Cancho-Grande B., Simal-Gándara J., 2012. Impact of phytosanitary treatments with fungicides (cyazofamid, famoxadone, mandipropamid and valifenalate) on aroma compounds of Godello white wines. *Food Chem.*, **131**, 826–836.

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 Chem.*, **117**, 529–537.

Gowman A.C., Picard M.C., Rodriguez-Uribe A., Misra M., Khalil H., Thimmanagari M., Mohanty A.K., 2019. Physicochemical analysis of apple and grape pomaces. *Bioresources*, **14**, 3210–3230.

Hammer Ø., Harper D.A., Ryan P.D., 2001. PAST, 2.04, Palaeontologia electronica, Norway.

Hou X., Xu Z., Zhao Y., Liu D., 2020. Rapid analysis and residue evaluation of six fungicides in grape wine-making and drying. *J. Food Compos. Anal.*, **89**, 103465.

IARC, 1993. Agents Classified by the IARC Monographs, Volumes 1-119. List of Classifications. Ochratoxin A, CAS No. 303-47-9. Available at: https://monographs.iarc.who.int/list-of-classifications (accessed on 02.09.2022).

IARC, 2018. Agents Classified by the IARC Monographs, Volumes 1–119. List of Classifications. 2,4-dichlorophenoxyacetic acid, CAS. No 94-75-7. Available at: https://monographs.iarc.who.int/list-of-classifications (accessed on 02.09.2022).

IBRAVIN, 2019. Ministério Público recebe novo pedido de suspensão do herbicida 2,4-D no RS, GZH: Brazil. Available at: https://gauchazh.clicrbs.com.br/economia/campo-e-lavoura/noticia/2019/11/mp-recebe-novo-pedido-de-suspensao-do-herbicida-24-d-no-rs-ck2qouch100pp01o0g360uxp0.html (accessed on 02.09.2022).

Jiang Y., Simonsen J., Zhao Y., 2011. Compression-molded biocomposite boards from red and white wine grape pomaces. *J. Appl. Polym. Sci.*, **119**, 2834–2846.

JMPR, 2006. Updating, the principles and methods of risk assessment MRLS for pesticides and veterinary drugs. Available at: http://www.fao.org/3/al932e/al932e.pdf (accessed on 02.09.2022).

Kochman J., Jakubczyk K., Janda K., 2021. Mycotoxins in red wine: Occurrence and risk assessment. *Food Control*, **129**, 108229.

Kumar P., Mahato D.K., Sharma B., Borah R., Haque S., Mahmud M.C., Shah A.K., Rawal D., Bora H., Bui S., 2020. Ochratoxins in food and feed: Occurrence and its impact on human health and management strategies. *Toxicon,* **187***, 151–162.*

Lewis K., Tzilivakis J., Green A., Warner D., 2006. Pesticide Properties DataBase (PPDB). Available at: http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (accessed on 02.09.2022).

Magistà D., Cozzi G., Gambacorta L., Logrieco A.F., Solfrizzo M., Perrone G., 2021. Studies on the efficacy of electrolysed oxidising water to control *Aspergillus carbonarius* and ochratoxin A contamination on grape. *Int. J. Food Microbiol.*, **338**, 108996.

Manjarres-López D.P., Andrades M.S., Sánchez-González S., Rodríguez-Cruz M.S., Sánchez-Martín M.J., Herrero-Hernández E., 2021. Assessment of pesticide residues in waters and soils of a vineyard region and its temporal evolution. *Environ. Pollut.*, **284**, 117463.

Meca G., Blaiotta G., Ritieni A., 2010. Reduction of ochratoxin A during the fermentation of Italian red wine Moscato. *Food Control*, **21**, 579–583.

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. *Int. J. Food Microbiol.*, **119**, 230–235.

Mello L.M.R., Machado C.A.E., 2021. Vitivinicultura brasileira: Panorama 2020. Comunicado Técnico - 223 da Embrapa Uva e Vinho, Bento Gonçalves, RS. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/227610/1/ComTec-223-21.pdf (accessed on 02.09.2022).

Munro I.C., Carlo G.L., Orr J.C., Sund K.G., Wilson R.M., Kennepohl E., Lynch B.S., Jablinske M., 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. *J. Am. Coll. Toxicol.*, **11**, 559-664.

Ndukwe J.K., Aliyu G.O., Onwosi C.O., Chukwu K.O., Ezugworie F.N., 2020. Mechanisms of weak acid-induced stress tolerance in yeasts: Prospects for improved bioethanol production from lignocellulosic biomass. *Process Biochem.*, **90**, 118-130.

OIV, 2021. World wine production outlook. OIV first estimates. Available at: https://www.oiv.int/public/medias/8726/en-oiv-2021-world-wine-production-first-estimates.pdf (accessed on 02.09.2022).

Oliva J., Martínez-Gil A.M., Lorenzo C., Cámara M.A., Salinas M.R., Barba A., Garde-Cerdán T., 2015. Influence of the use of fungicides on the volatile composition of Monastrell red wines obtained from inoculated fermentation. *Food Chem.*, **170**, 401–406.

Pan X., Dong F., Liu N., Cheng Y., Xu J., Liu X., Wu X., Chen Z., Zheng Y., 2018. The fate and enantioselective behavior of zoxamide during wine-making process. *Food Chem.*, **248**, 14–20.

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. *Anal. Bioanal. Chem.*, **389**, 1697–1714.

Petruzzi L., Corbo M.R., Baiano A., Beneduce L., Sinigaglia M., Bevilacqua A., 2015. In vivo stability of the complex Ochratoxin A–*Saccharomyces cerevisiae* starter strains. *Food Control*, **50**, 516–520.

Petruzzi L., Corbo M.R., Sinigaglia M., Bevilacqua A., 2014. Yeast cells as adsorbing tools to remove ochratoxin A in a model wine. *Int. J. Food Sc. Technol.*, **49**, 936–940.

Pichon V., Charpak M., Hennion M.C., 1998. Multiresidue analysis of pesticides using new laminar extraction disks and liquid chromatography and application to the French priority list. *J. Chromatogr. A.*, **795**, 83–92.

Remiro R., Irigoyen A., González-Peñas E., Lizarraga E., de Cerain A.L., 2013. Levels of ochratoxins in Mediterranean red wines. *Food Control*, **32**, 63–68.

Rifai A., Souissi Y., Genty C., Clavaguera C., Bourcier S., Jaber F., Bouchonnet S., 2013. Ultraviolet degradation of procymidone–structural characterization by gas chromatography coupled with mass spectrometry and potential toxicity of photoproducts using in silico tests. *Rapid Commun. Mass Spectrom.*, **27**, 1505–1516.

Romanazzi G., Feliziani E., 2014. *Botrytis cinerea* (Gray Mold). *In: Postharvest decay*: *Control Strategies*. 131–146. Bautista-Baños, S. (ed.), Academic Press, London.

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. *Aust. J. Grape Wine Res.*, **25**, 316–326.

Schusterova D., Hajslova J., Kocourek V., Pulkrabova J., 2021. Pesticide residues and their metabolites in grapes and wines from conventional and organic farming system. *Foods*, **10**, 307.

Tempère S., Marchal A., Barbe J.C., Bely M., Masneuf-Pomarede I., Marullo P., Albertin W., 2018. The complexity of wine: Clarifying the role of microorganisms. *Appl. Microbial.* Biotechnol., **102**, 3995–4007.

Tıraş Z.Ş.E., Okur H.H., Günay Z., Yıldırım H.K., 2022. Different approaches to enhance resveratrol content in wine. *Ciência Téc. Vitiv.*, **37**, 13–28.

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.

Ulrih N.P., Skrt M., Košmerl T., Wondra M., Abram V., 2020. Part I. Polyphenols composition and antioxidant potential during ‘Blaufränkisch’ grape maceration and red wine maturation, and the effects of trans-resveratrol addition. *Food Chem. Toxicol.*, **137**, 111122.

Yoon J.Y., Kim J.E., Song H.J., Oh K.B., Jo J.W., Yang Y.H., Lee S.H., Kang G., Kim H.J., Choi Y.K., 2021. Assessment of adsorptive behaviors and properties of grape pomace-derived biochar as adsorbent for removal of cymoxanil pesticide. *Environ. Technol. Innov.*, **21**, 101242.

Yousefi M., Khorshidian N., Mortazavian A.M., 2021. Detoxification properties of microorganisms in foods. *In: Microbial Biotechnology in Food and Health*. 81–112. Academic Press, India.