The fate of *Fusarium* mycotoxins (deoxynivalenol and zearalenone) through wort fermenting by *Saccharomyces* yeasts (*S. cerevisiae* and *S. pastorianus*).

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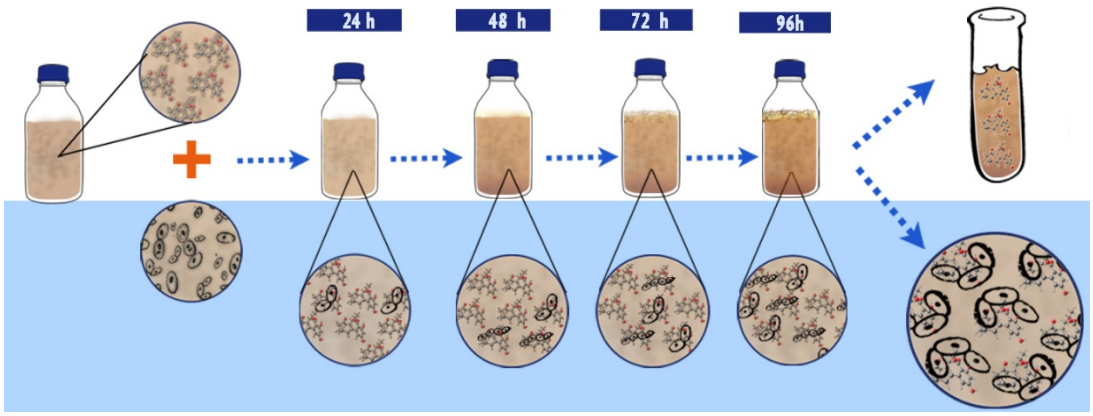
ABSTRACT

The aim of this study was to evaluate the effect of 15 commercial yeasts in the mitigation of the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) during the brewing process. *Saccharomyces* strains (10 strains of *S. cerevisiae* and 5 of *S. pastorianus*) were used to ferment DON and ZEN contaminated wort. Wort samples were taken every 24 h during fermentation, while mycotoxin analysis in yeast was performed at the end of fermentation (96 h); additionally, pH and ethanol content were measured daily. For mycotoxin analysis, after immunoaffinity purification of sample extracts, analysis was performed using an Ultra-High-Pressure Liquid Chromatograph coupled with a diode array or fluorescence detector (UHPLC-DAD/FLD). Mycotoxin presence had no significant effect on the ethanol production during brewing. At the end of fermentation, 10–17 % of DON and 30–70 % of ZEN had been removed, 6 % of the initial concentration of DON and 31 % of the ZEN being adsorbed by the yeast. Beermakers must pay careful attention to the raw material since a high percentage of DON could be present at the end of the beer fermentation process. Future studies should focus on the quantification of "masked" mycotoxins that are relevant to food security.



HIGHLIGHTS|

- After beer fermentation, 83-90 % of DON and 30–70 % of ZEN in wort was detected.
- DON and ZEN adsorption ability of *S. pastorianus* and *S. cerevisiae* was evaluated.
- *Saccharomyces* yeasts show higher adsorptions for ZEN that for DON.
- DON and ZEN contamination of wort had no effect on ethanol production.

Keywords: Beer fermentation; *Fusarium* mycotoxins; *Saccharomyces* yeast; cell wall, mycotoxin adsorption.

1. INTRODUCTION

Beer is one of the most popular alcoholic beverages in the world (FAO, 2009). In 2017, the global beer production increased to about 1.95 billion hectoliters up from 1.3 billion hectoliters in 1998 (Statista, 2019). It is obtained by fermenting the boiled wort (water extract of malt cereal) using yeast (Walther, Ravasio, Qin, Wendland, & Meier, 2015). Cereals used in beer production can be contaminated by different mycotoxins: barley and wheat by ochratoxin A (OTA), trichothecenes, (such as, deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins), and zearalenone (ZEN). Corn used as adjunct during mashing, is proven to be mainly contaminated by fumonisins (FBs) and aflatoxins (AFs), but also by trichothecenes (Sanchis, Marín, & Ramos, 2013). All these mycotoxins have been associated with human and animal diseases, some affect the immune system (trichothecenes), while others are hepatotoxic (FBs), nephrotoxic (OTA), estrogenic (ZEN), teratogenic, mutagenic and carcinogenic (AFs). (IARC, 1993; Leblanc, Tard, Volatier, & Verger, 2005; Panel & Chain, 2014; Vila-Donat, Marín, Sanchis, & Ramos, 2018).

Various studies detected high frequencies and levels of mycotoxins in barley crops (Kroes et al., 2002; Piacentini, Savi, Olivo, & Scussel, 2015; Rodríguez-Carrasco, Fattore, Albrizio, Berrada, & Mañes, 2015). Their transfer from barley to malt and to beer subsequently would be expected due to their known resistance to the physical treatments applied during the brewing process (Hazel & Patel, 2004; Inoue, Nagatomi, Uyama, & Mochizuki, 2013; Wolf-Hall, 2007). However, recent surveys of mycotoxins in beer produced in different geographical regions have mainly detected low levels of DON and his masked

forms (DON3G, 3ADON), ZEN, OTA and FBs (in the order of µg/L) (Bertuzzi, Rastelli, Mulazzi, Donadini, & Pietri, 2011; Pascari, Ortiz-Solá, Marín, Ramos, & Sanchis, 2018; Peters et al., 2017; Wall-Martínez, Pascari, Ramos, Marín, & Sanchis, 2019). These small concentrations, together with the available studies, suggest that the various stages of the brewing process (malting, mashing, wort boiling and fermentation) could exert a mitigation effect on mycotoxin contamination or lead to a transformation or degradation of these molecules (Inoue et al., 2013; Kroes et al., 2002; Lancova et al., 2008).

A study on the transfer of *Fusarium* mycotoxins previously performed by our research group proved that malting could lead to a decrease in the level of DON (from 46.6 % up to 78.8 %) and an increase in deoxynivalenol-3-glucoside (DON3G) level, while ZEN remained almost unchanged (Pascari, Gil-Samarra, Marín, Ramos, & Sanchis, 2019). Also, DON and its metabolites showed a high transfer from malt to the wort during mashing (30 % - 60 %). During mashing and boiling FBs were reduced by 50 to 100 %, and ZEN reduction was higher than 89 % (Pascari, Rodriguez-Carrasco, et al., 2019). Considering that the investigated mycotoxins were not completely eliminated after mashing and boiling, there is a need to continue studying the mycotoxin levels through the fermentation process.

Many species of acid lactic bacteria, yeast and fungi have been shown to enzymatically bind, remove or biodegrade mycotoxins (Faucet-Marquis, Joannis-Cassan, Hadjeba-Medjdoub, Ballet, & Pfohl-Leszkowicz, 2014; Fuchs et al., 2008; Haskard, El-Nezami, Kankaanpää, Salminen, & Ahokas, 2001). It is known that *Gluconobacter oxydans* is able to degrade more than 90 % of patulin (PAT) 80 % of OTA and 27 % of aflatoxin B_1 (AFB_1) (Markov et al., 2019). Yeast biomass have shown the ability to adsorb mycotoxins due to the presence of mannoproteins and β-glucans in their cell wall, *based on physical adsorption, ion exchange and complexation,* (Faucet-Marquis et al., 2014; Huwig, Freimund, Käppeli, & Dutler, 2001; Shetty & Jespersen, 2006). in this sense, Campagnollo et al. (2015) utilized an *vitro* test to determine the capacity of one strain of *S. cerevisiae* to bind mycotoxins: ZEN (77 %), OTA (13 %), aflatoxin B1 (AFB_1) (38 %) and DON (17 %) at pH 6, and ZEN (75 %), OTA (59 %), AFB_1 (48 %) and DON (11 %) at pH 3.

Few studies are available investigating the fate of mycotoxins during beer fermentation. Garda et al. (2005) identified a 53 % reduction of DON and T-2 toxin at the end of the fermentation process with a strain of *Saccharomyces cerevisiae*. Nathanail et al. (2016), using a strain of *Saccharomyces pastorianus* observed a mycotoxin reduction of up to 15 % for DON, 17 % for DON-3-G, 34 % for HT-2, and 31 % for T-2 toxins after a 96 h period of beer fermentation. From the abovementioned studies,

it can be seen that different *Saccharomyces* strains have different ability in binding mycotoxins. Also, considering the physical nature of the adsorption, this result could be modulated by different physical parameters, such as pH and temperature, that are applied during the process. (Avantaggaito, Solfrizzo, & Visconti, 2005; Huwig et al., 2001). Therefore, the present study focuses on evaluating under single conditions the effect of different starter cultures (10 *S. cerevisiae* and 5 *S. pastorianus* strains) in the mitigation of the *Fusarium* mycotoxins (DON and ZEN) contamination during the beer fermentation process.

2. MATERIALS AND METHODS

2.1 Reagents and chemicals

Water was obtained from a Milli-Q® SP Reagent water system from Millipore Corp. (Brussels, Belgium). Methanol and acetonitrile (HPLC grade) were purchased from Scharlab (Sentmenat, Spain). Mycotoxin standards of DON and ZEN were purchased from Romer Labs (Tulln, Austria).

Immunoaffinity columns (IAC) for DON (DONPREP®) and ZEN (EASY-EXTRACT® ZEARALENONE) clean-up were purchased from R-Biopharm (Rhone LTD Glasgow, UK). Phosphate buffer saline (PBS) was prepared with potassium chloride (0.2 g) (Panreac, Castellar del Vallès, Spain), potassium dihydrogen phosphate (0.2 g) (98–100 %, Panreac, Castellar del Vallès, Spain), disodium phosphate anhydrous (1.16 g) (99 %, Panreac, Castellar del Vallès, Spain) and sodium chloride (8.0 g) (> 99.5 %, Fisher Bioreagents, New Jersey, USA) in 1 L of Milli-Q water. The pH was brought to 7.4 (Basic 20, CRISON) with hydrochloric acid 1 M. Bacteriological peptone was purchased from Biokar Diagnostics (Allonne, France), magnesium sulfate heptahydrate was acquired from Probus SA (Badalona, Spain) and agar powder from VWR Prolabo (Leichestershire, UK). Yeast extract peptone dextrose medium (YEPD) was prepared with 10 g/L of yeast extract powder (Bacto, Madrid, Spain), 20 g/L of peptone (Biokar Diagnostics, Allone, France), 20 g/L of glucose (Fisher chemical, UK) and 20 g/L of agar (Prolabo, Leuven Belgium).

2.2 Preparation of mycotoxins solutions

DON concentration in the stock solution was checked by UV spectroscopy according to AOAC Official methods of analysis, chapter 49 (AOAC, 2005) obtaining a concentration of the stock solution 758 and

844 µg/mL, respectively. Standard solutions of DON and ZEN were prepared in methanol at a concentration of 10.0 mg/mL and stored at 4 °C. Calibration curves were prepared by appropriate dilution of known volumes of the stock solution with the mobile phase.

2.3 DON and ZEN contamination of malted barley

Twenty kg of malted barley (*Hordeum vulgare*) were supplied by a malting plant (Malteria la Moravia S.L., Bell-lloc d'Urgell, Spain) in March 2018. DON and ZEN were not detected in malted barley by UHPLC-DAD/FLD analysis. A control batch of malted barley was used to prepare control wort (absent of DON and ZEN), the rest of the malt was contaminated using a toxigenic strain of *Fusarium graminearum* (F.46) (producer of DON and ZEN) obtained from the collection of strains of the Food Technology Department of the University of Lleida, Spain. Barley grains were disinfected according to Pascari, Rodriguez-Carrasco, et al. (2019). Briefly, 500 g of grains were submerged into 0.4 g/100 mL sodium hypochlorite solution for 2 min and then abundantly rinsed twice with sterile distilled water. Then the grains were placed in hermetically closed sterile ISO bottles and left overnight at 4 °C with a small amount of water to allow the water activity to reach a value close to 0.99 (Aqualab Series 3 TE, Decagon Devices Inc., Washington, USA). Malted barley was aseptically transferred to Petri dishes and 1 mL of a spore suspension of *F. graminearum* (10^6 spores/mL) was transferred to each dish. Petri dishes were incubated at 25 °C for 30 days, then, contaminated grains were dried at 40 °C, homogenized, and DON and ZEN content was determined. The final concentrations in the contaminated malt was 4600 µg/kg and 2800 µg/kg for DON and ZEN, respectively.

2.4 Yeast

Fifteen freeze-dried yeast strains of various commercial brands, 10 *S. cerevisiae* (ale-high fermentation) and 5 *S. pastorianus* (lager-low fermentation) were used. All the yeasts used throughout this study were purchased from a website specialized in the sale of beer preparation supplies (www.cervezasdelmundo.com).

2.5 Wort production

Mycotoxin contaminated wort was prepared by adding appropriate proportions of the contaminated grains previously produced to uncontaminated malted grits. Maceration was carried out mixing 2.5 L of deionized water with 500 g of malted barley (previously ground) and then maintaining the mix for 15 min at 45 ºC, followed by 1 h at 65 ºC, ending with 72 ºC for another 15 min. The wort was decanted,

and the density was adjusted to 1005-1010 kg/m^3 with deionized water. The wort was boiled for approximately 2 h (up to a density of 1050 kg/m^3) and stored in sterile bottles until fermentation.

2.6 Fermentation

Fermentation assays were performed at 250 mL scale with triplicate samples. The process lasted 96 h at 20 ºC. The concentration of yeast used to inoculate the wort was selected following the brand's instructions. Yeast concentration was adjusted at 10^6 CFU/mL for all the wort samples. An initial count was made using a Thoma cell counting chamber. Moreover, a confirmatory initial yeast count on YEPD medium was performed for all the samples. Briefly, 1 mL of samples were diluted from 10^{-1} to 10^{-7} with sterile saline peptone. Then, 100 µL of each dilution were superficially spread on YEPD agar medium dishes and incubated at 25 °C for 48 h.

Two samples were taken daily in the following time points: 24 h, 48 h, 72 h, and 96 h. The first sample (10 mL) was used to determine the pH, and alcohol content (with a portable densimeter DMA 35, Anton Paar). The second sample (30 mL) was centrifuged at 1869 x g for 10 min to eliminate the yeast from the fermented wort and was used for mycotoxins determination. After four days' fermentation, the yeast was separated from the remaining wort by centrifugation at 1869 x g for 10 min, lyophilized, weighted and all samples (wort and yeast) were stored at -18 ºC until HPLC analysis.

2.7 Mycotoxin extraction

DON and ZEN were extracted using specific immunoaffinity columns (DONPREP® and EASY EXTRACT® ZEARALENONE, respectively) following the manufacturer's instructions.

2.7.1 DON extraction in malt

Five grams of malt previously ground with an IKA® A11 Basic mill (Darmstadt, Germany) were mixed with 1 g of sodium chloride and 40 mL of Mili Q water in a 250 mL Erlenmeyer flask, followed by 30 min stirring. Then, samples were centrifuged for 10 min at 1846 x g. Supernatant was filtered through a glass microfiber paper filter (Whatman nº 1, Maidstone, UK) and 2 mL of the filtrate was passed through the immunoaffinity column. The column was then washed with 10 mL of bi-distilled water and the toxins were eluted with 3 mL of methanol HPLC grade (the first 1.5 mL performing back-flushing). Samples were evaporated under a low nitrogen stream at 40 °C and resuspended in mobile phase (acetonitrile:methanol:water, 5:5:90, v/v/v). Every resuspended extract was filtered through a nylon filter (0.4 µm) before being injected (50 µL) into the UHPLC-DAD system.

2.7.2 ZEN extraction in malt

Five grams of malt previously ground with an IKA® A11 Basic mill (Darmstadt, Germany) were mixed with 25 mL of extraction solvent acetonitrile:distilled water (75:25, v/v) and stirred for 30 min. Samples were centrifuged for 10 min at 1846 x g and 10 mL of the supernatant was mixed with 40 mL of phosphate buffer saline (PBS) at pH 7.4 The obtained 50 mL were passed through the immunoaffinity column which was afterwards washed with 20 mL of PBS. ZEN was eluted with 3 mL of acetonitrile (the first 1.5 mL performing back-flushing). Samples were evaporated under a low nitrogen stream at 40 °C and resuspended in 1 mL of acetronitrile:Mili Q water (50:50). Every resuspended extract was filtered through a nylon filter (0.4 µm) before being injected (50 µL) into the UHPLC-FLD system.

2.8 Mycotoxins analysis in wort

2.8.1 DON extraction in the fermented wort

Wort was filtered through a glass microfiber filter paper (Whatman no. 1, Whatman, Maidstone, UK) and 1 mL of filtered sample was loaded onto an immunoaffinity column at a flow rate of 2 mL per minute. The column was washed by passing 10 mL of Mili Q water at a rate of 5 mL per minute, and finally, air was allowed to pass through the column to remove the residual liquid. DON elution was performed by adding 1.5 mL + 1.5 mL of methanol, performing a back-flushing each time. The samples were evaporated and resuspended in 1 mL of mobile phase for UHPLC-DAD analysis.

2.8.2 ZEN extraction in the fermented wort

20 mL of centrifuged wort was mixed with 20 mL of PBS, and pH was adjusted to a pH of 7.4. 20 mL of the mixture was passed through an immunoaffinity column at a flow rate of 2 mL per minute, the column was washed by passing 10 mL of water at a rate of 5 mL per minute and finally, air was allowed to pass through the column to remove the residual liquid. The elution was performed by adding 1.5 mL + 1.5 mL of acetonitrile, back-flushing being performed each time. The samples were evaporated and resuspended in 1 mL of acetronitrile:Mili Q water (50:50) for UHPLC-FLD analysis.

2.9 DON and ZEN extraction in yeast

The DON and ZEN extraction in yeast was performed following the Campagnollo et al. method (Campagnollo et al., 2015). Two hundred milligrams of lyophilized yeast were suspended in 2 mL of 0.1M potassium phosphate buffer pH 6.5, mixed on a rotating shaker (Infors AG CH-4103 Bottmingen,

Switzerland) for 60 min at 25 °C and sonicated for 15 min (Brason 2800). The samples were centrifuged (Eppendorf, Hamburg, Germany) at 1846 x g for 10 min at room temperature and 0.8 mL of the supernatant was removed and analyzed by UHPLC. Negative controls (200 mg of yeast in buffer solution) were also prepared and analyzed.

2.10 UHPLC system

The determination of DON was performed using an Agilent Technologies 1260 Infinity UHPLC system (California, USA) coupled with an Agilent 1260 Infinity II Diode Array Detector (DAD). A Gemini® C18 column from Phenomenex 150x4.6 mm (California, USA) with a particle size of 5 µm and a pore size of 110 Å was used. Absorption wavelength was setup at 220 nm. The mobile phase was composed of methanol:acetonitrile:water Mili Q (5:5:90, v/v/v) and set at a flow rate of 1 mL/min. For ZEN determination, the same equipment and column were used but the HPLC was couplet to an Agilent 1260 Infinity Fluorescence detector (FLD) (excitation and emission wavelengths were 274 nm and 455 nm, respectively). The mobile phase was acetonitrile:Milli Q water (60:40 v:v) with pH adjusted to 3.2 with acetic acid. Flow rate was set at 0.6 mL/min. The column temperature was 40 °C, the injection volume was 50 µL and total run time was 20 min for both mycotoxin analyses.

2.11 Validation of analytical methods

Selectivity was checked by injecting 50 µL of standard solution for at least three times (150 µg/L) and comparing retention time and peak resolution between injections. For linearity check, a calibration curve of eight concentration levels for each toxin (20, 30, 50, 100, 250, 500, 1000, 3000 µg/L for DON and 30, 50, 100, 300, 500, 1000, 1500, 3000 µg/L of ZEN solutions) was prepared and injected into the system, generating a linear regression plotting solutions' concentration versus peak area. Finally, precision was evaluated preparing blank wort and yeast samples spiked with DON and ZEN at several concentration levels and recovery percentages were determined: 75–90 % (wort), 67–71 % (yeast) for DON, and 84–97 % (wort), 73–80 % (yeast) for ZEN (Table 1). The limit of detection (LOD) was considered as three times the signal to noise ratio. Method performance was assessed according to Commission Regulation (EC) 401/2006 (European Commission, 2006) (Table 1).

2.12 Statistical Analysis

All the results were presented as the average of triplicate tests and expressed as mean ± standard deviation. Statistical evaluations were performed by one-way analysis of variance (ANOVA) and Tukey test ($p = 0.05$) using Minitab 18 software.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the wort

Control and mycotoxin contaminated wort had an initial density of 1.043 g/cm^3, whereas initial pH was 4.85 (control) and 5.0 (mycotoxin contaminated). Initial mycotoxin contamination of wort was 1380 µg/kg (DON) and 600 µg/kg (ZEN). Neither DON nor ZEN were detected in the control wort.

3.2 Alcohol production and pH

Final pH and alcohol produced after 96 h of wort fermentation for the different yeast strains assayed are shown in Table 2.

The pH of the wort at the end of fermentation ranged between 3.80-4.34 (control) and 3.75-4.15 (contaminated). There are no reports of an effect on pH due to the presence of mycotoxins, so the variation in the pH observed in control and contaminated wort could be attributed to the initial difference since pH of the control wort was slightly lower than that of the contaminated wort. Regarding alcohol production, the results agree with those reported by Nathanail et al. (2016), the mycotoxins present in the wort had no significant effect on alcohol production during beer fermentation. The progress of alcohol content during the fermentation of the wort using one *S. cerevisiae* and one *S. pastorianus* strain is showed in Figure 1.

Figure 1 shows that in the four sampling points analyzed during 96 h of beer fermentation no significant differences were found in ethanol content between contaminated and uncontaminated wort.

3.3 Deoxynivalenol fate

Mycotoxin adsorption on the cell wall structure is an interaction between the toxin and the functional groups of the cell surface. Yeast cell wall contains many different adsorption sites, mainly represented by polysaccharides (glucans and mannans), proteins and lipids (Faucet-Marquis et al., 2014).

Considering that mycotoxins adsorption is of physical nature (based on ion exchange and complexation) (Huwig et al., 2001), mycotoxin contamination has proven to present little influence on yeast activity (Nathanail et al., 2016). There are few studies dedicated to investigate the adsorption of DON to yeast cell wall, most of them being performed *in vitro* (Campagnollo et al., 2015; Scott, Kanhere, Daley, & Farber, 1992; Sørensen & Sondergaard, 2014). The present work assesses the adsorption of DON onto brewing yeast cell wall. Table 3 presents DON concentration in yeast and brewing wort throughout the 96 h of fermentation.

The highest effect in DON reduction occurred during the first 24 h, resulting in 84 % to 95 % of the initial content of mycotoxin that remained in the must. The final concentration of DON in the obtained beers, after 96h of fermentation, ranged from 97.9-82.5% of the initial contamination, with reductions higher than 15% in 9 out of 15 beers. Similar results were obtained by Nathanail et al. (2016) who reported a reduction of 15 % in the DON content in a wort using an *S. pastorianus* yeast strain. Garda et al., (2005) observed a reduction of 53.3% in malt spiked with DON and ZEN. Previously, Scott et al. (1992) using a contaminated wort with DON and ZEN after 7-9 days of fermentation with *S.* cerevisiae reported that DON was stable through the process. The variability in DON reduction in the different studies can be due to various factors including pattern of grain infection, process parameters, interactions with the yeast used or mycotoxin metabolization in a modified form as DON3G, 3ADON and 15ADON (Bryła, Ksieniewicz-Woźniak, Waśkiewicz, Szymczyk, & Jędrzejczak, 2018; Kostelanska et al., 2011; Nathanail et al., 2016). Also, from the table 3 it can be seen that the total concentration that was identified in the beer and in the yeast residues suggests that approximately 10% of toxin could be transformed by the yeasts into metabolites, most probably into 3-acetyl-deoxynivalenol (Khatibi et al., 2011). This suggests the need in a deeper biological analysis of the yeast strains used in beer fermentation in order to better evaluate the nature of this change.

Regarding DON content in yeast, up to 6.36 % of the initial content in the wort was detected. Mycotoxins binding to the yeast is attributed to the formation of both, hydrogen bonds and van der Waals interactions between hydroxyl, ketone, and lactone groups from β-glucans of the cell wall and the mycotoxin (Faucet-Marquis et al., 2014; Shetty & Jespersen, 2006; Takata et al., 2010; Yiannikouris et al., 2006). In 3 of the 15 samples, the adsorbed mycotoxin was found to be below the detection limit, and the wort fermented by these yeasts presented the lowest decrease in DON after 96 h of fermentation (<11 %). In similar works, Campagnollo et al. (2015), performing an *in vitro* test,

determined a DON binding in *S. cerevisiae* of 11.6 and 17.6% at pH 3 and 6.5, respectively, while Nathanail et al. (2016) found quantifiable DON values in the yeast pellet analyzed after wort fermentation with a high DON initial concentration (10,000 µg/L). The difference in adsorption levels could be explained by strain-dependent cell wall structure, the phase of the cell cycle and growth conditions such as pH, temperature, oxygenation rate, and concentration and nature of carbon source (Jouany, Yiannikouris, & Bertin, 2005).

3.4 Zearalenone fate

The ZEN concentration in yeast and brewing wort throughout 96 h of fermentation is presented in Table 4. Unlike DON, whose degradation occurred mainly during the first 24 h, the reduction in the ZEN content was continuous during the 96 h of fermentation. The beer wort showed a final ZEN reduction between 31 and 72 %, and the toxin adsorbed to yeast ranged between 4.5 and 31.3%.

In similar studies, Campagnollo et al. (2015) established the ability of the beer fermentation residue to bind ZEN at acid and neutral pH, being *S. cerevisiae* able to adsorb 75.1 % (pH 3) and 77.5 % (pH 6.5) of the initial contamination. Previously, Yiannikouris et al. (2004) examined four different compositions of β-(1,3)-D-glucans and β-(1,6)-D-glucan for their ability to adsorb ZEN at pH 3.0, 6.0 and 8.0. At neutral and acid pH, ZEN adsorption ranged from 53.8 to 76.9 % (pH 3.0) and from 57.9 to 73.9% (pH 6.0), but at basic pH (8.0) the adsorption ranged from no detected (ND) to 51.8 %. In our study, the difference in mycotoxin binding cannot be attributed to the pH differences between the samples, as the pH of the samples ranged only from 3.8 to 4.2.

It is known that the ZEN adsorption capacity of the yeast cell wall is strongly correlated with cell wall β-D-glucans content; additionally, differences between the binding of mycotoxins and yeast could vary due to the charge distribution, chemical nature of mycotoxin regarding the surface properties, geometry and the available surface of the adsorbent (Bakutis, Baliukonienė, & Algimantas, 2005; Kabak, Dobson, & Var, 2006). In addition to the adsorption to the yeast, the reduction in the ZEN content can be attributed to a possible biodegradation or metabolization of ZEN to other compounds, as β-zearalenol (β-ZEL) and α-zearalenol (α-ZEL) by the brewing strains of *Saccharomyces* during beer production (Keller et al., 2015; Lancova et al., 2008; Mizutani, Nagatomi, & Mochizuki, 2011). This added to the reductions during the malting and maceration (higher than 50 %)(Pascari, Gil-Samarra, et al., 2019;

Pascari, Rodriguez-Carrasco, et al., 2019), could explain the low or no incidence level reported in studies of ZEN in beers (Pascari et al., 2018; Wall-Martínez et al., 2019).

3.5 *Saccharomyces cerevisiae* vs *Saccharomyces pastorianus*

Table 5 shows the yeast performance for the two *Saccharomyces* species in the context of mycotoxin adsorption evaluated in the present study.

In our study fermentation temperature was 20 °C, more suitable for *S. cerevisiae*, resulting in a higher quantity of biomass for this yeast after 96 h of fermentation and, consequently, a higher ethanol production; however, this increase in biomass is hardly significant ($p = 0.037$) and did not mean a significant impact on adsorption for DON and ZEN mycotoxins. These result in the need to study the process variables (temperature, concentration of yeast) to optimize the removal of mycotoxins during the fermentation process for *S. cerevisiae and S. pastorianus yeast*.

It is relevant to expand the study to the different forms of DON and ZEN, with the aim of identifying if that percentage of mycotoxin that was not detected in wort or yeast that could be "masked" and be in a sufficient concentration to represent a toxicological risk to the consumer.

4 CONCLUSION

The effect of 15 *Saccharomyces* yeasts in the mitigation of *Fusarium* mycotoxins (DON and ZEN) through brewing was studied, performing a comparative analysis between *S. cerevisiae and S. pastorianus.* ZEN showed higher adsorption (4.50 % - 31.25 %) that DON (LOD - 6.36 %) on *Saccharomyces* yeast cells. On the other hand, mycotoxin content decreased at the end of fermentation between 11-17% for DON and 31-72% for ZEN in the wort. There was no significant difference in the decrease of DON or ZEN when is fermented with *S. cerevisiae* or *S. pastorianus* at 20 °C. Even though mycotoxin presence had no significant effect on ethanol production at the end of the fermentation, brewers must pay special attention to mycotoxin content in raw materials since a low concentration could be present in the beer or even in the yeast (for unfiltered beers) at the end of brewing process.

Future studies should focus the analysis of "masked" mycotoxins in wort and yeast to determinate if the reduction in the contamination was due to a transformation, to elimination, or to binding to the yeast and finally, on the optimization of process conditions, e.g. yeast concentration and process temperature, in order to improve the decrease of mycotoxin content during the beer production process.

ACKNOWLEDGEMENT

The authors are grateful to the University of Lleida (JADE Plus 218/2016 grant), to the Spanish Ministry of Economy and Competitiveness (MINECO, AGL2014-55379-P project) for funding this work. Wall-Martínez HA is grateful to the Consejo Nacional de Ciencia y Tecnología (CONACyT) from Mexico for the scholarship.

CONFLICTS OF INTEREST

The authors declare have no conflicts of interest to disclose, have full control of all the primary data and accept to allow the journal to review their data if they are requested.

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