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Alcoholic fermentation effects on malt spiked with trichothecenes

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Abstract

Malt contaminated with DON and T-2 toxin at levels of between 0 and 1000 ng/g was used for fermentation on a laboratorial scale in order to evaluate the decontamination caused by alcoholic fermentation using *Saccharomyces cerevisiae*. Fermentation was carried out over a period of 120 h at 14 °C and a reduction in the levels of contamination was detected by GC. Forty one percent of the initial quantity of DON and T-2 toxin contamination was passed on to the final liquid portion. Six percent of the initial contamination was detected in the wort, which signifies a total decontamination of 53% of DON and T-2 toxin, taking into account both the wort and the filtered sample.

Keywords: Decontamination; Trichothecenes; Fermentation; Deoxynivalenol; T-2 toxin; *Saccharomyces cerevisiae*

1. Introduction

Trichothecenes are products of the secondary metabolism of moulds of the *Fusarium*, *Myrothecium*, *Trichothecium*, *Cephalosporium*, *Verticimosporium* and *Stachybotrys* genera. They can be produced by microorganism moulds pre- and post-harvesting, at temperatures between 0 and 35 °C and relative humidity between 80% and 90%. These contaminated cereals are the main sources of non-macrocyclic trichothecenes in food and animal feed, especially cereal grains such as barley, wheat, corn and rice (Mello et al., 1999; Schapira et al., 1989; Soares & Rodriguez-Amaya, 1989).

Trichothecenes are relatively stable during most processing and are difficult to remove from moderately contamined grains (Niessen & Donhauser, 1993). Deoxynivalenol (DON) is the group B trichothecene most frequently detected by researchers. Another is T-2 toxin, a group A trichothecene, that has high toxicity and is often found in foods (Ueno, 1983, 1986).

Several authors such as Smith et al. (1990), Scott (1984, 1993), Bennet and Richard (1996), among others have been studying detoxification procedures for these mycotoxins. Detoxification using physical, chemical or microbiological procedures may have the effect of destroying, modifying or absorbing these mycotoxins, thus reducing or eliminating their toxic effects. The physical processing of grains can reduce the levels of mycota and their contaminating metabolites. Polishing and damp grinding stand out among these processes, but their results vary depending on the mycotoxin and procedure being studied (Lee, Lee, Park, & Ueno, 1992). During the fermentation process used in the making of bread from wheat grains contaminated with deoxynivalenol, levels of mycotoxin, especially OTA were shown to be reduced (Niessen & Donhauser, 1993).

Scott (1992) mentioned experiments of alcoholic fermentation by *Saccharomyces cerevisiae* using a malt contaminated with deoxynivalenol and zearalenone in which the results after 7–9 days of fermentation showed that DON was stable to the process. Of the initial content of zearalenone, 69% was converted into β-zearalenol (β-ZEL) and 8.1% into α-zearalenol. Most of the metabolization of the zearalenone occurred on the first and second days of fermentation, thus showing the instability of this mycotoxin during fermentation. The yeast *Saccharomyces cerevisiae* reduced levels of T-2 toxin and DAS during alcoholic fermentation (Flannigan, Day, Douglas, & McFarlance, 1985).

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According to Bennet and Richard (1996), DON was not completely destroyed by the alcoholic fermentation process and could be detected in high levels in both the solid residue and the fermented liquid. The same applied to zearalenone and fumonisin. These results suggest that other procedures, such as separation by density, treatment with chemical reagents or even washing with water, should be carried out on grains used as raw materials for fermentation and also that studies of the effect of fermentation on decontamination should be better evaluated.

Another yeast, *Candida lipolytica*, reduced the level of T-2 toxin in the growth medium by up to 12% of the initial quantity in 23 days (Whitehead & Flannigan, 1989).

This study aims to evaluate detoxification by alcoholic fermentation using *S. cerevisae* in malt artificially contaminated with DON and T-2 toxin in combined concentrations with levels varying from 0 to 1000 ng/g.

2. Materials and methods

2.1. Malt

Malt collected at a brewery in Rio Grande do Sul state, Brazil was used. Malt was evaluated for zearalenone, ochratoxin A, and aflatoxins B_1, B_2, G_1 and G_2 occurrence by thin layer chromatography as described by Soares and Rodriguez-Amaya (1989). The screening for the trichothecenes DON and T-2 toxin was carried out using gas chromatography in a procedure which used a second extraction as described by Badiale-Furlong and Soares (1995) and derivation with TFAA as described by Nunes (2003).

2.2. Yeast

The yeast came from a pure culture of a selected strain of *Saccharomyces cerevisiae* for rapid fermentation (522 Davis strain, Montrachet, University of California, Perdomini brand) was used at the recommended dose of 30 g/h l.

2.3. Mycotoxin standards

Standards of DON and T-2 toxin from the Sigma Chemical Company were dissolved in benzene: acetonitrile (95:5), and their concentrations were determined spectophotometrically in a Varian spectrophotometer of the Cary 100 model by Bennett and Shotwell (1990). The final standard concentrations were 50 μg/ml.

2.4. Contamination of malt

A treatment table for the central composite desing experiment (surface response method) (CCD) was set up to study the effect of alcoholic fermentation by *Saccharomyces cerevisiae* on DON and T-2 toxin levels. To do this, 80 g of finely ground malt (⩾28 mesh) was contaminated at the respective levels with standards of DON and T-2 toxin and left at room temperature for 24 h before the wort was prepared. The contamination range was from 0 to 1000 ng/g for each toxin. $N_f = 2^2$ treatments were decided upon for the factorial portion, $n_\alpha = 2.2$ for the axial portion and $n_c = 4$ at the central point $(0;0)$, totalizing 12 treatments (Table 1).

2.5. Fermentation

The wort was prepared by adding water to the contaminated malt in a 1:10 ratio of malt:water following the procedure described in Fig. 1. The fermentation was conducted in a 2 l erlenmeyer.

The wort was cooled and 0.3 g/l of yeast was added. It was then heated to 40 °C for 15 min to activate the yeast. Fermentation was carried out in an incubator chamber at 14 °C for 120 h and samples were collected every 12 h in order to follow the fermentation process. When fermentation had finished, the samples were filtered with a qualitative filter paper and the contamination levels were evaluated in the filtered sample and the dry wort. The experiment was repeated on three separate occasions.

2.6. Determination of trichothecenes in the solid residue

The residue was dried in an oven at 80 °C with aeration for 6 h. After the trichothecenes DON and T-2

Table 1
CCD planning to determine the effect of decontamination on DON and T-2 toxin in alcoholic fermentation

Treatment	X_1	X_2	DON (ng/g)	T-2 (ng/g)
1	−1	−1	146	146
2	1	−1	854	146
3	−1	1	146	854
4	1	1	854	854
5	0	0	500	500
6	0	0	500	500
7	−1.414	0	0	500
8	1.414	0	1000	500
9	0	−1.414	500	0
10	0	1.414	500	1000
11	0	0	500	500
12	0	0	500	500

$X_1 = (\xi_1 - 500)/354$ and $X_2 = (\xi_2 - 500)/354$, where ξ_1 is the contamination value of DON (ng/g) and ξ_2 is the contamination value of T-2 toxin (ng/g). The value 354 (500/1414) is the value of α for CCD (for 2 factor).