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Biotransformation of trichothecene mycotoxins during fermentation with lager yeast

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Introduction

The fate of mycotoxins and especially modified mycotoxins (also known as masked mycotoxins) during malting and brewing has recently gained attention. Modified mycotoxins are mainly conjugation products created through detoxification mechanisms of living organisms. During fermentations microbes may transform mycotoxins to less toxic compounds (decontamination) or deconjugate modified mycotoxins present in raw materials (activation). Thus, malting and brewing may alter mycotoxins chemically. This study aimed to investigate the metabolic fate of *Fusarium* trichothecenes including HT-2, T-2, deoxynivalenol (DON) and its modified form deoxynivalenol-3-glucoside (D3G) during fermentation by lager yeast.

Materials and methods

A lager yeast was cultivated in triplicate shake flasks containing 100ml of 11.5°Plato unhopped wort containing mycotoxins at different concentrations or in different combinations. Wort and yeast biomass samples were taken periodically over a 4-day cultivation and analysed by liquid chromatography-triple quadrupole-mass spectrometry (LC-TQ-MS) and LC-quadrupole-time of flight (Q-Tof)-MS to examine the kinetics of these compounds during fermentation and potential metabolic products formed by yeast cells (Fig. 1).

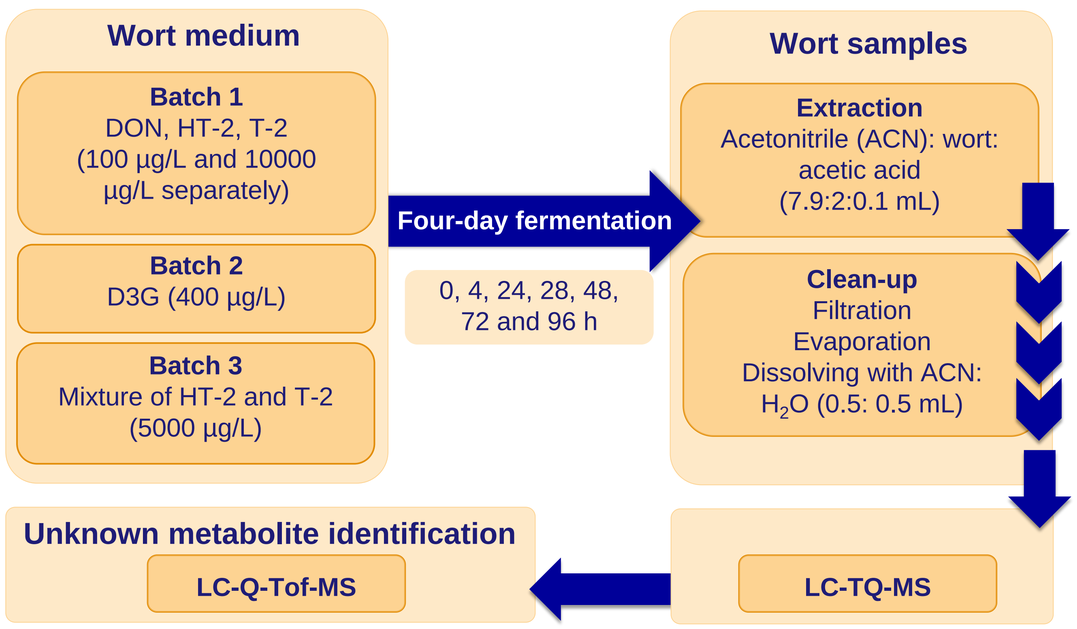

Figure 1. *Experimental procedure*

Liquid chromatography –mass spectrometry (LC-MS)

- ✓ **Column**: Waters Atlantis T3 (3.0 mm × 150 mm, 3 µm)
- ✓ **Eluents**: (A) H_2O +10 mM ammonium acetate, (B) ACN
- ✓ **Kinetic study apparatus:** Waters Xevo ESI-TQ-MS
- ✓ **Metabolomic study apparatus:** Waters Premier Q-Tof-MS (full scan from *m/z* 100-1000)

Results

Yeast was tolerant to these mycotoxins at concentrations up to 10 mg/l. Thus, the presence of trichothecenes, even at levels far in excess of those found in naturally contaminated barley, had little or no effect on sugar utilisation, alcohol production, pH, yeast growth or cell viability (data not shown).

This study revealed that D3G was chemically stable during fermentation and was not deconjugated to DON. In fact, yeast was able to glucosylate DON present in wort to D3G during fermentation (verified with standard measurements). As seen in Fig 2., a faster concentration decrease was observed in the samples spiked with 100 µg/L HT-2 and T-2 compared to DON and D3G. At 10 mg/L level (high) T-2 and HT-2 might already influence yeast functionality explaining different kinetic profiles. Results of the samples treated with a mixture of HT-2 and T-2 showed a similar profile to those individually inoculated with HT-2 and T-2 (data not shown).

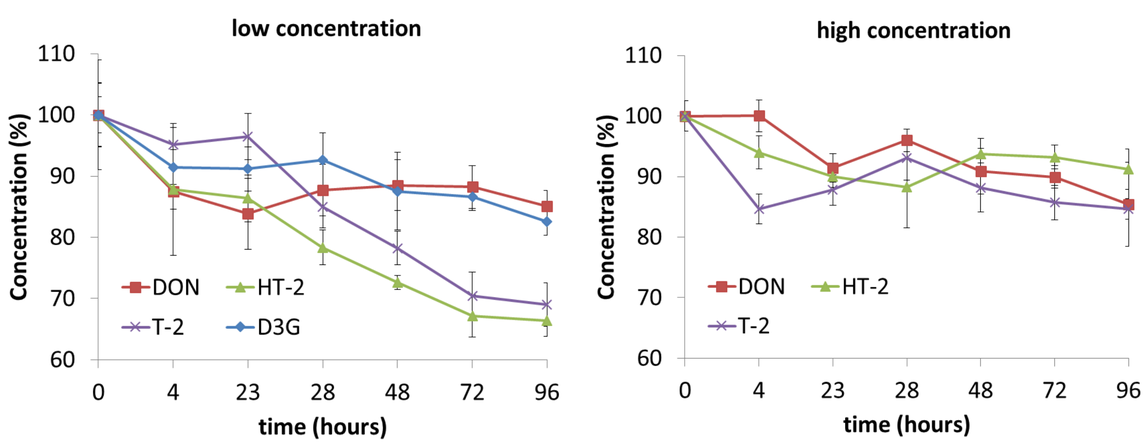

Figure 2. *Time-course kinetics of HT-2, T-2, DON and D3G inoculated in low concentration (100 µg/L of DON, HT-2, T-2 and 400 µg/L of D3G) and high concentration (10000 µg/L of DON, HT-2, T-2) in wort samples during four-day fermentation by lager yeast (mean concentration % ± SD, n=6)*

Several potential metabolites were annotated and further confirmed by LC-Q-Tof-MS/MS analysis (Table 1). Acetylation and de-acetylation of mycotoxins were the main metabolic pathways. HT-2 was detected to be the major metabolite of T-2 biotransformation in lager yeast, as has been reported in other organisms.

Table 1*. Trichothecene metabolites in wort samples after four-day fermentation by lager yeast. Metabolite identification on-going (more metabolites expected to be found, manuscript in preparation)*

Samples	Metabolites	Formula	*m/z* found	Molecular ion	RT (min)	Abundance	ppm error
DON	D3G	$C_{21}H_{30}O_{11}$	517.1934	$[M+CH_3COO]^-$	6.08	After 23 h	1.42
	Acetyl-DON	$C_{17}H_{22}O_7$	337.1245	$[M+H]^-$	7.18	After 4 h	12.6
HT-2	Acetyl-HT-2	$C_{24}H_{34}O_9$	484.2535	$[M+NH_4]^+$	12.82	After 4 h	-0.2
T-2	HT-2	$C_{22}H_{32}O_8$	463.1727	$[M+K]^+$	10.75	After 4 h	-1.7
	Acetyl-T-2	$C_{26}H_{36}O_{10}$	526.2639	$[M+NH_4]^+$	14.52	After 4 h	-0.5

Conclusions

- ✓ Yeast may alter the concentration or form of the mycotoxins present, thereby influencing their toxicity
- ✓ Approximately 10-40% of toxins were biotransformed by yeast during four-day fermentation
- ✓ D3G was not modified by yeast during fermentation
- ✓ Novel modified forms of trichothecene metabolites were detected, revealing that (de)acetylation and glucosylation were the main metabolic pathways
- ✓ **Microbial processing may alleviate toxicity of mycotoxins, thereby providing new perspectives for development of mitigation strategies**