Barley fungi and their mycotoxins in beer production

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Short CV

Anneleen Decloedt graduated in 2011 as M. Sc. Biochemistry and Biotechnology, Ghent University. In 2014 she was the project manager of the Beer4Dreams team that won the first and public price at the Belgian Ecotrophelia competition and the fourth price at the European competition. She successfully defended her PhD in public health and food safety at the Laboratory of Chemical Analysis, Ghent University in 2015. Since January 2015 she has been working as a research fellow at the Laboratory of Biochemistry and Brewing of Ghent University and University College Ghent, regularly presenting her work in scientific papers and at international congresses.

Abstract

Decreased barley crop yield and quality is often the result of pathogenic fungi that originate from the contaminated seed, the wind or the soil (e.g. from crop residues). Well-known fungal diseases of barley are powdery mildew, blotch, leaf spot, rust, stripes disease, ergot and *Fusarium* head blight (FHB). FHB is caused by strains of several *Fusarium* species and favored by humid conditions during flowering and early stages of kernel development.

Fusarium fungi can produce many different toxic metabolites (mycotoxins) such as trichothecenes (e.g. deoxynivalenol = DON), fumonisins, zearalenone and enniatins. Mycotoxins are considered to be the most dangerous chronic-toxic contaminants present in the human diet. The tolerable daily intake dose (TDI) of the main *Fusarium* mycotoxins are between <0.06 and <2 µg/kg body weight. For T-2 and HT-2 for example a consumer can already exceed the total permitted daily intake dose with the consumption of one liter of beer

containing 3 to 5 µg/L of the toxins. In addition, a variety of modified forms of mycotoxins occur which can lead to an underestimation of the true amount of mycotoxins in foodstuffs. In beer, glycosylated mycotoxins can be expected that can be released from the grains during the brewing process. For the malting and brewing industry it remains a challenge to produce malt and beer with the lowest possible levels of mycotoxins and their modified forms.

Samples from the entire processing from grains up to the final beer were analyzed with LC-MS/MS for 18 (modified) mycotoxines. The quantitative multi-mycotoxin analysis method was optimized for both liquid, solid and mixed samples to a limit of quantification of 0.03 - 53 ppb, depending on the type of mycotoxin.

In beers of eight different Belgian beer types, produced in 2014 or 2015, rather low concentrations of mycotoxins were found, mainly DON, DON-3-glucoside (DON-3G), enniatine (ENN) and (modified) zearalenon (ZEN) metabolites. Despite these low concentrations, a probabilistic risk analysis revealed that consumers of fruit beer can be at risk for mycotoxin HT-2.

Malting did not lead to a clear increase in mycotoxins, even not for the five experimental cases were spores of *Fusarium* strains were pitched at the beginning of the germination of the barley and outgrowth of the fungus occurred. An incubation of milled pale malt with the same five *Fusarium* strains during four days also resulted in visual growth of the fungus, but without significant mycotoxin increases. It was concluded that mycotoxin production takes place mainly on the field, probably because during malting the fungi are not stressed by limited resources.

The pilot and industrial brews, which were followed up for mycotoxin content by sampling at 11 to 13 process points, revealed that water-soluble mycotoxins like α-zearalenol are released from the milled malt and stay present up into the final beer. The less water-soluble mycotoxins like zearalenone mainly remain in the spent grains. DON and DON-3G show intermediate behavior.