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PhD Review Report

PhD thesis „**Mycotoxin contamination of spring barley in the chain barley grains–final products**"
authored by **Ing. Alexadra Malachova PhD**

The objective of the thesis is the description of fate of various *Fusarium* mycotoxins during beer production. The author focused on the presence of mycotoxins in all stages of the process and observed the relation between contamination in raw material and corresponding intermediates and final products. This was enabled by mastering advanced targeted analytical methods using combination of liquid chromatography with mass spectrometry detection. Proper methods were validated and used for various matrices and resulted in extensive screening of final products (beers) available directly on the markets worldwide.
In order to understand the changes in content of particular mycotoxins, the author focused further on modifications of molecular structure of the compounds of interest either as a result of plant metabolism, or as a result of acidic/alkaline/enzymatic conditions that may occur during processing. This aim requested involvement of the latest techniques available for detection of small molecules and their structure elucidation – liquid chromatography coupled to high-resolution mass spectrometry.
While focusing mostly on the well-known Fusarium mycotoxins frequently found in cereal products in substantial amount, the author also focused on survey of the marginal mycotoxin pentahydroxyscirpene and its acetylated form.

The thesis is structured as a set of nine peer-reviewed publications in highly rated scientific periodicals. Here I consider important to mention that their content has undergone a blind-review and has been found to be sufficiently innovative while based on the experiments that respect the principles of good research practice. The collection of publications is properly framed within an accompanying text that clearly describes the contribution of the author to the general knowledge of the problematic of *Fusarium* mycotoxin. The thesis is precisely written and the experiments themselves very well documented in appendices. There are no typing errors and the language is clear and concise.

The publication in **Appendix I** is a multidisciplinary study on pentahydroxyscirpene. Authors contribution consist of LC/MS analyses of naturally contaminated barley samples. Surprisingly, the publication lacks "Conclusions" section. Can the author provide us with conclusions that have been made from her results?

The publication in **Appendix II** describes the detoxification process of deoxynivalenol in wheat isogenic lines with different resistance level to Fusarium infection. Structure characterization of deoxynivalenol metabolites revealed eight products assigned to two metabolic pathways. The author presumes that the cleavage of DON conjugates may contribute to DON burden in mammal's gastrointestinal tract.

The publication in **Appendix III** summarizes the metabolism of HT-2 and T-2 toxin in barley with 9 and 13 metabolites of HT-2 and T-2 respectively with HT-2-β-3GLc being the major metabolite.

The publication in **Appendix IV** presents the survey of deoxynivalenol, nivalenol, T-2 and HT-2 toxin content in barley. The effect of fungicide, weather, cultivar and presence/absence of hull has been evaluated, but the conclusion of relationship between barley and malt contamination has not been provided due to the small scale of experiment.

The publications in **Appendix V** and **VI** deal with the deglycosylation of the most common deoxynivalenol plant metabolite DON-3-glucoside. The publication in **Appendix V** deals with chemical ways of cleavage using acidic and basic condition with the prospect of simple indirect method for DON-3-glucoside determination. The author concludes that those methods, although previously reported, does not provide valid results due to the easily degradable structure of DON molecule. The publication in **Appendix VI** follows up and offers enzymatic method for deglycosylation at mild conditions that do not damage deoxynivalenol molecules.

The publications in **Appendix VII**, **VIII** and **IX** are devoted to the analyses of deoxynivalenol and its conjugates in final product, i. e. beer.
The publication in **Appendix VIII** presents the development of LC-MS/MS method. Most of the study is dedicated to the method optimization and validation and the method is finally applied to a small set of 60 beer samples. The method was carefully optimized and properly validated.
The publication in **Appendix IX** is based on the same method applied to the large sample set of 374 beers. The author concluded that the majority of the samples did not did not pose a toxicological risk for consumers, anyway, several highly contaminated beers could cause exceeding of tolerable daily intake. The publication listed in **Appendix VII** is a review summarizing the methods used for the determination of mycotoxins in beer. It is based on the knowledge gained in previous publications of the same authors and completes them with comprehensive overview of methods used by other researchers discussing their pros and cons.

At this point, I would suggest listing the last three publications chronologically, as they form a great illustration of the time-course of the scientific work on single topic: starting with method validation, going on with extensive exploitation of optimized method, culminating with comprehensive review. Putting the review in front of the other two is not decreasing the scientific value of the thesis, but it is distracting to the reader.

Questions:

- As mentioned above - can the author provide us with conclusions that have been made from her results of pentahydroxyscirpene analyses presented in Appendix I?
- As author reported the occurrence of PHS in naturally infected barley samples, is it possible estimate the importance of PHS to the toxicity of Fusarium mycotoxin cocktail in cereal based food?
- The author says (page 31, paragraph 2) that the mycotoxin contamination increases during malting and brewing due to the release of additional amount of mycotoxin. Is not it possible that the additional increase is also caused by wet and warm condition during the process enabling the growth of fungi itself?
- The relationship between barley and malt contamination has not been provided in Appendix III due to the small scale of experiment. Are there any additional recent data (either own or reported) available nowadays?
- Is it possible, that some mycotoxins pass from cereals to the cereal products easier than the others? Why?

Conclusion:

The reviewed thesis fulfils all requirements for obtaining PhD degree and it is ready to be defended in front of respective scientific committee. I would like also to highlight an extensive amount of novel knowledge and outstanding scientific activity confirmed by numerous papers published in highly ranked scientific journals. Therefore, I recommend the thesis to be accepted.

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