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Effect of cultivation and processing on *Fusarium* mycotoxins in malting barley

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Abstract

The study investigated the contamination of malting barley grain with mycotoxins of *Fusarium* during the process of growth and development of grain and its subsequent processing in the malting plant into manufactured beer. Attention was focused on their hygienic quality, zearalenone and deoxynivalenol content was determined by HPLC. None of the samples exceeded the legal limits. The experiment evaluated the influence of cultivar (Francin, Bojos), the effect of nitrogen fertilization rate and fungicide treatment. Francin appeared more resistant to ZEA occurrency, while Bojos was more resistant to DON occurency. Nitrogen fertilization rates of 80 and 110 kg/ha may have caused a higher susceptibility of the crop to *Fusarium* attack. The hypothesis that mycotoxin susceptibility would increase with increasing fertilization rate was not supported. The environment of the malt house may also have contributed to the increase in mycotoxin content. In the case of fungicide application, it cannot be clearly assessed whether it affected the mycotoxin content. The field experiment was adversely affected by bad weather. Differences ($P < 0.05$) were found when monitoring the ZEA and DON content of Bojos and Francin grain samples. The ZEA content of Bojos and Francin malt were not different ($P > 0.05$). Significant differences ($P < 0.05$) were found when monitoring the DON content of Bojos malt samples, the DON content of the Francin malt was comparable ($P > 0.05$). The Bojos beer samples were not different in ZEA and DON content ($P > 0.05$). For Francin beer, there were differences ($P < 0.05$).

Keywords: malting barley grain; malt; beer; mycotoxins; deoxynivalenol; zearalenone; hygienic food quality

1 Introduction

Barley (*Hordeum vulgare*) is considered as a crop with a great potential. Of the total barley production, about 75% is used for food purposes, mainly for malting. Around 20% of production is used as feed and the remaining percentage is used in other sectors. Malting barley is a key raw material for the production of beer, and together with hops, yeasts and water forms its basis. The characteristics of malt and beer are determined by the production process, including, among others, the operations of malting, mashing-in, mashing, lautering, wort boiling, hopping, fermentation, maturation, filtration, stabilization and packaging (Martiník et. al, 2023; Pascari et al., 2018).

A large number of microorganisms can be found in barley grain, malt and beer, which are indicative of their safety and quality. The main problem is the occurrence of mould. Health problems are mainly caused by their ability to produce dangerous mycotoxins, which may not be removed by the processing process itself. Fungi can infect the crop during cultivation in the field, or contamination can occur during poor storage, transport or processing into the final product (Rausch et al., 2020; Freire and Sant'Ana, 2018; Barkai-Golan and Pater, 2008; Benett and Klich, 2003). Mycotoxins in the diet is a worldwide problem for the human population, which is exposed to mycotoxins primarily through consumption of contam-

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inated food commodities (Malíř et al., 2023; Pustjens et al., 2022). Maximum limits for mycotoxin content in foodstuffs are set in Commission Regulation (EU) 2023/915, as amended. For the production of mycotoxins, it is necessary that moulds have suitable conditions for growth. Due to detoxification processes, dangerous 'masked' forms of mycotoxins can also be found in cereals. Filamentous fungi of the genus *Fusarium* are important pathogens of cereals. Their presence in malting barley can result in changes in the characteristics of the barley itself, such as reduced germination, discolouration and beer aftertaste. In the Czech Republic, the pathogens *Fusarium graminearum* and *F. culmorum*, which produce these dangerous mycotoxins, are most common (Pascari et al., 2018; Havránková and Ovesná, 2012; Wolf-Hall et al., 2007; Berthiller et al., 2005). Trichothecenes and fumonisins are considered to be important fusarium mycotoxins in barley grain. These toxins are chemically and thermally stable, for this reason they can overcome the cooking process to some extent and thus transfer from malt into beer (Piacentini, 2019; Creppy, 2002).

Zearalenone (ZEA) is a fusarium toxin. There is evidence that ZEA and its metabolites have estrogenic activity. In addition, it has hepatotoxic, haematotoxic, immunotoxic and enotoxic effects (Malíř et al., 2023; Magan and Olsen, 2004). Deoxynivalenol (DON), referred to as vomitoxin, is also one of the trichothecene mycotoxins produced by the genus *Fusarium*. DON plays a key role in immunological disorders and nervous system problems. Due to the fact that, mycotoxins can cause acute and chronic poisoning in humans, it is necessary to prevent their formation in food. In addition to the above-mentioned adverse effects of fusarium mycotoxins, their presence also presents technological difficulties in beer production. Mycotoxins can inhibit enzyme synthesis during the malting process. Furthermore, they are involved in the slow growth of yeasts during beer production and may be responsible for gushing (Freire and Sant'Ana, 2018; Edwards, 2004). As beer is the most consumed alcoholic beverage in the European Union, control of its hygienic quality and healthiness is essential (Malíř et al., 2023; Bauer et al., 2016).

In this study were used spring barley cultivar Bojos and Francin. It is a malting cultivar intended for the production of 'Czech Beer' according to Research Institute of Brewing and Malting. Bojos is a semi-late cultivar of medium stature, which gives a consistently high grain and foreground yield in all areas. This cultivar is resistant to pathogens such as downy mildew (gene *mlo*), rust and leaf spot. Francin is a semi-arable cultivar, with a high proportion of forward grain, medium stem length and exceptional resistance to lodging. Francin is characterised by high yields in the beet and maize area. It is also characterised by its high resistance to rusts and stalk breakage, good resistance to mildew, downy mildew and leaf spot. Like the Bojos cultivar, it meets the malting quality criteria for 'Czech beer' (Psota, 2024). The aim of this work is to assess the risks of processing grain contaminated with fusarium mycotoxins. The main objective is to capture the dynamics of changes in mycotoxins during processing in the malting plant and their subsequent transfer to the finished product. Different doses of nitrogen fertilization are also considered in the experiment. Furthermore, crop treatments such as fungicide application and targeted infection with the pathogen *Fusarium culmorum* were used. It is assumed that with increasing nitrogen doses will increase the susceptibility of the crop to fungal attacks.

2 Materials and methods

The experiment was based on the cultivation of 12 variants of malting barley grain samples of the Bojos and Francin cultivars, followed by the processing of the grain into malt and the production of beer

2.1 Growing conditions for barley

The aim was to evaluate the hygienic quality of the grain, malt and beer produced. A field experiment was set up in Velká Bystřice (275 m a.s.l.) near Olomouc, Czech Republic (49.60315, 17.35465). It is a climatic region of moderately warm, moderately humid with medium heavy soil, the soil type in the locality is Cambisol, the average annual temperature is 10.7 °C and the average monthly rainfall is 53.0 mm. Seeding took place in the month of March 2023. Attention was also paid to the effect of different levels of nitrogen fertilization, targeted grain infection with *F. culmorum*, and grain treatment with the fungicide Prosaro (0.75 l/ha). *F. culmorum* has lower temperature requirements, the optimum for its growth is 21 °C. The active substances of Prosaro are prothioconazole 125 g/l and tebuconazole 125 g/l. The nitrogen fertilization doses were 50, 80 and 110 kg/ha. The yield potential of barley is high in this area and based on previous experience was known that fertilizing with 50 kg/ha of nitrogen is not enough, so were chose 3 levels of fertilization to have a comparison.

2.2 Infection of barley by Fusarium culmorum

The presence of the *F. culmorum* pathogen (KM16902, VUKROM, Kroměříž, Czech Republic) in grain from the Agricultural Research Institute Kroměříž (VUKROM) was confirmed using a Bürker's chamber. This infected grain was shaken in water and then the crop of our field experiment was treated with this spray. Infection with *F. culmorum* was carried out at flowering time on the part of the crop untreated with Prosaro fungicide and also on treated crop variants. Infection must have taken place in the morning hours under heavy dew (an ideal condition for the development of a fungal infestation). Our experiment was affected by unfavourable conditions in June – high temperatures and low rainfall (average temperature in June was 19.8 °C and rainfall in June were 8.4 mm). On the other hand, there was heavy rainfall in early August. On 13 August 2023, the barley grain was harvested. A total of 12 variants were analysed depending on the treatment from each cultivar (Table 1).

Table 1 *Scheme of the field experiment*

Identification of the sample variant (Bojos or Francin)	Nitrogen fertilization (kg/ha)	Application of fungicide Prosaro	Infection Fusarium culmorum
1	50	–	–
2	80	–	–
3	110	–	–
4	50	–	YES
5	80	–	YES
6	110	–	YES
7	50	YES	–
8	80	YES	–
9	110	YES	–
10	50	YES	YES
11	80	YES	YES
12	110	YES	YES

2.3 Malting process of barley

From all the harvested malting barley grain variants (Bojos, Francin cultivars) malts were produced in the micro-malting plant (Ravoz, Czech Republic). The equipment is three-phase (steeping, germination and kilning), the contents of each sample were 1 kg. Steeping and germination were carried out at 13 °C (Table 2), kilning was carried out according by the temperature program 55 °C 6 hours, 60 °C 6 hours, 65 °C 8 hours, 78 °C 3 hours, 20 °C 1 hour, in total 24 hours. In view of possible mould contamination between each batch of barley, the steeping box was always sanitized with a 0.5% peroxyacetic acid solution.

2.4 Beer production

The beer was produced in a laboratory equipment with a final wort volume of 20 litters (Royal Catering, Prague, Czech Republic), which also includes a lautering tun equipped with a shower. The mashing was carried out with constant stirring. The temperature and time program of all phases of beer production is described in Table 3. Hops, Saaz (granules type 90, Svoboda-Fraňková, Czech Republic) with a bitterness of 4.1–5.2% α-bitter acids were added to the wort. The resulting bitterness in IBU was calculated to be 25, and the dosage of hops was adjusted to this value in relation to its α-bitter acid content. The bitter wort was aerated using an aeration candle from a laboratory compressor equipped with a HEPA filter. The wort was inoculated with *S. cerevisiae* RIBM 95 (Research Institute of Brewing and Malting, Czech Republic) and after 5 days of main fermentation at 11 °C, the fermented wort was shock cooled to 1 °C and the racked green beer was placed for 21 days in a refrigerator for maturation in PET bottles. All beer samples were produced by the infusion method.

2.5 Sample preparation and preconcentration of DON and ZEA

Grain samples were milled using an MEZOS FQC 109 (Czech Republic) laboratory mill. 25 g of the sample were weighed into a 50ml ground glass (Erlenmeyer) flask, and 100 ml of a 70% methanol solution was added. The flask was placed in an ultrasonic bath for 15 minutes. After the addition of 100 ml of deionized water, the sample was centrifuged at 4,000 rpm for 10 minutes (Hettich® Universal 320, Merck, Germany). The supernatant was then filtered through a glass frit (S3), and 10 ml of the filtrate was transferred to an immunoaffinity column (StarLine™ MultiStar™) that had been conditioned with 20 ml of demineralized water. The sample was passed through the column using a SPE Extra system (Chromservis, Czech Republic) at a flow rate of 1 ml/min. After washing the column with a phosphate buffer, analytes were eluted with 2 ml of a 50% methanol solution. The excess methanol was evaporated under a stream of nitrogen at 40 °C, and the residue was re-dissolved in 1 ml of a 50% methanol solution prior to analysis. For beer samples, 50 ml of degassed beer (with the pH adjusted to 7.2 using NaOH) was used. This volume was directly applied to the immunoaffinity column and processed as described above.

2.6 HPLC/MS analysis of mycotoxins

The analysis was performed on an HPLC system (Agilent 1260, USA) coupled to a mass detector (Agilent 6460, USA) with electrospray ionization (ESI). Separation was carried out on an ARION® Polar Plus C18 column (3.0 µm, 150 mm × 4.6 mm) equipped with a guard column (4.0 mm × 3.0 mm, 3.0 µm) (Chromservis, Czech Republic). The mobile phase consisted of methanol and 5 mm ammonium acetate, and the gradient elution program was applied as specified in Table 4. The mobile phase flow rate was set to 0.5 ml/min, and the column temperature was maintained at 40 °C. ESI was operated in the negative ion mode ($[M–H]^-$) with the following conditions: capillary voltage of –5.0 kV, source temperature of 180 °C, desolvation temperature of 400 °C, nebulizer gas flow of 40 l/h, and desolvation gas flow of 60 l/h. Helium was used as the collision gas.

For Multiple Reaction Monitoring (MRM), the transitions were as follows:
For DON: Precursor ion at m/z 355 with product ions at m/z 295 and m/z 265, using a collision energy of 17 eV.
For ZEA: Precursor ion at m/z 317 with product ions at m/z 273 and m/z 299, using a collision energy of 22 eV.

Calibration curves were constructed using standard solutions at concentrations of 10, 20, 50, 100, 200, 500, and 1000 ng/ml.

Validation parameters:
For DON:
Calibration curve:
$y = 11181x + 46614$, $R^2 = 0.9989$
For barley and malt:
LOD = 4.8 µg/kg and LOQ = 15.8 µg/kg
For beer:
LOD = 59.6 ng/l and LOQ = 196.1 ng/L
Mean recovery (by standard addition): 97.3 ± 5.7%
For ZEA:
Calibration curve: $y = 8606x + 16556$, $R^2 = 0.9986$
For barley and malt:
LOD = 3.6 µg/kg and LOQ = 11.9 µg/kg
For beer: LOD = 44.1 ng/l and LOQ = 146.5 ng/L
Mean recovery (by standard addition): 95.1 ± 7.2%

Methodology of the work and experimental measurements on the already established method were carried out according to Pernica et al. (2019). The measurement of mycotoxin content by HPLC was performed in 2 replicates. The average and standard deviation were calculated from the measured values. Data were statistically processed by the Shapiro-Wilk test for assessing normality and compared by one-factor ANOVA in Excel based on the resulting P-value, at the 5% significance level ($P < 0.05$). Sample 1 was used as a blank to determine the content of a given mycotoxin for each commodity (grain/malt/beer) and variety (Bojos/Francin).

Table 2 *Malting scheme*

Malting stage		hours	temperature
Steeping	1st immersion	6	13
	1st air rest	6	13
	2nd immersion	6	13
	2nd air rest	8	13
	3nd immersion	5	13
	3nd air rest	10	13
	4nd immersion	2	13
	4nd air rest	12	13
Germination	germination	144	13
Kilning	pre-drying	6	55
	intermediate drying	6	60
	curing	8	65

Table 3 *Temperature and time program of all phases in beer production*

Brewing process	Temperature (°C)	Time (min)
Mashing	40	5
Proteolysis	52	15
I. Sugar-forming temperature	62	30
II. Sugar-forming temperature	72	30
Mash-out	90	10
Pumping to the settling tank	90	5
Settling	85	30
Pumping of the sweet wort	80	30
Sweetening	80	10
Boiling with hops	100	90
I. Hop	100	0
II. Hop	100	40
III. Hop	100	75
Whirling of bitter wort	95	5
Hop lees settling	95	30
Pumping into the fermentation tank	11	30

Table 4 *Mobile phase gradient elution procedure*

Time (min)	0.05 M Amonium acetate	Methanol (%)
0	80	20
6	20	80
6.1	80	20
12	80	20

3 Results and discussion

Research aimed at eliminating the entry of mycotoxins into beer based on field experiments, monitoring the dynamics of mycotoxin growth in the malthouse and evaluating the intensity of the transition into beer is desirable. Moulds and their metabolites are still a persistent problem in the brewing industry. It is necessary to monitor their occurrence in beer and commodities used for its production. This is also agreed in many scientific studies (Malíř et al., 2023; Martiník et al., 2023; Piacentini, 2019; Pascari et al., 2018; Bauer et al., 2016). The identification of each sample and its treatment is explained above in Table 1, where further details can be found. The results of the ZEA content in grain, malt and beer for each sample variant are presented in Table 5. The values obtained from the determination of the DON content are given in Table 6.

Table 5 *Zearalenone (ZEA) content in barley grain, malt and beer*

Bojos / number of the sample	1	2	3	4	5	6	7	8	9	10	11	12
Barley grain (µg/kg)	3.2	4.8	5.1	14.3	17.9	12.4	11.3	25.4	22.1	14.6	5.3	9.7
Malt (µg/kg)	8.4	6.8	6.7	10.4	12.7	16.4	11.9	18.4	53.4	21.6	9.8	8.7
Beer (µg/l)	0.14	0.18	0.06	0.08	0.11	0.04	0.09	0.04	0.21	0.19	0.14	0.11
Francin / number of the sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Barley grain (µg/kg)	6.6	3.4	4.1	5.8	5.9	4.1	6.2	5.4	6.4	4.7	5.8	6.9

Table 6 *Deoxynivalenol (DON) content in barley grain, malt and beer*

Bojos / number of the sample	1	2	3	4	5	6	7	8	9	10	11	12
Barley grain (µg/kg)	32.4	44.7	35.9	57.8	51.7	43.9	28.7	95.4	34.8	68.7	61.8	39,7
Malt (µg/kg)	43.7	63.4	51.7	47.3	68.9	72.4	42.6	96.7	54.7	57.8	66.7	42,4
Beer (µg/l)	0.35	0.43	0.51	0.12	0.19	0.17	0.35	0.64	0.75	0.42	0.14	0,54
Francin / number of the sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Barley grain (µg/kg)	21.7	68.9	47.6	43.2	84.7	57.8	36.1	112.4	57.3	106.7	104.9	41.8
Malt (µg/kg)	35.1	69.8	75.8	67.2	84.6	69.2	61.9	135.7	68.3	111.9	124.7	157.9
Beer (µg/l)	0.19	0.25	0.36	0.28	0.67	0.41	0.34	0.18	0.39	0.24	0.29	0.63

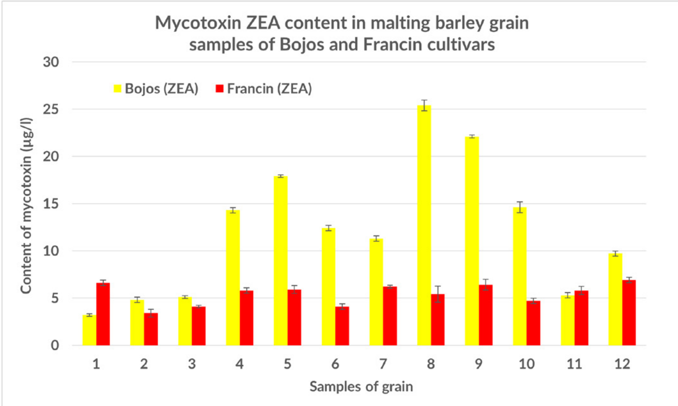
3.1 *Determination of the mycotoxin zearalenone (ZEA) in grain, malt and beer*

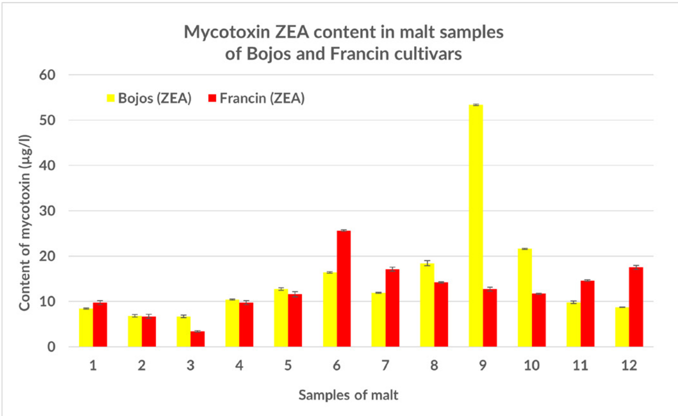
According to Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in foodstuffs and repealing Regulation (EC) No 1881/2006, as amended, the maximum limit for the presence of ZEA in grains of unprocessed cereals is 100 µg/kg. Figure 1 shows the ZEA content of the grain in the Bojos and Francin cultivars. The highest value of ZEA in the grain of the Bojos cultivar was measured in the variant of sample 8. Statistically significant differences ($P < 0.05$) were observed among the grain samples of the Bojos cultivar. Significantly lower values of ZEA content were found in the Francin samples. This is probably a factor of the cultivar characteristics and its resistance to mould infestation. Statistically significant differences in ZEA content ($P < 0.05$) were found between the different variants of grain samples of the Francine cultivar. None of all the monitored samples exceeded the permitted limit for ZEA content in grain of unprocessed cereals according to the above mentioned legislation.

Figure 2 shows the ZEA content of the malt. No limits are set for malt in Commission Regulation (EU) 2023/915. However, malt has been found to contain significantly higher levels of ZEA than grain. The malting process of already contaminated barley provides favourable conditions for the growth of moulds and their dangerous mycotoxins. This may be due to the warm and humid malting environment during the germination phase of the grain (Papadopoulou et al., 2000; Schwarz et al., 1995). Wolf-Hall (2007) indicates that there are factors that influence the concentration of mycotoxins at different stages of malting. For example, a given fungal strain, its concentration and vigour, the location of mycelia or spores and the relationships between organisms (synergism, antagonism). When comparing the malts, the cultivars did not show as much variance in ZEA content as the grain samples. Although the highest grain ZEA content was observed in sample 8 of the Bojos cultivar

(Figure 1), for malt the highest value was found in sample 9. No statistically significant differences were found between the malt samples for the Bojos cultivar ($P > 0.05$). In the case of the cultivar Francin, the highest ZEA content result was measured in the variant of sample 6. However, no statistically significant differences ($P > 0.05$) were found even among the samples of the malt cultivar Francin. For the variant samples of Francin 4, 5, 6 and 10, 11, 12 were can observe an increasing content of ZEA in malt with increasing nitrogen fertilization rate. The opposite trend was observed for sample variants 1, 2, 3 and 7, 8, 9. With increasing nitrogen fertilization rate, the content of ZEA increased in the case of Bojos malt in sample variants 4, 5, 6 and 7, 8, 9. An interesting result was also found when comparing the ZEA content of Bojos malt between the variants of samples 7, 8, 9, which were treated with fungicide Prosaro, compared to the variants of samples 1, 2, 3, which were not treated, and yet the ZEA content of these samples was lower. Although the use of the pesticide should prevent fungal growth and therefore the production of mycotoxins, the effect of the fungicide could have been influenced by many other factors – the chosen active ingredient, the method and time of application of the product or climatic and weather conditions (Šíp et al., 2007).

Dangerous mycotoxins can be transferred from infected raw materials to malt and then to beer. Moulds and their spores will not get into the beer. The brewing process acts as a decontamination method and the moulds and spores are destroyed at the latest at the hop brewing stage. However, some mycotoxins survive the process due to their thermal stability (Wolf-Hall, 2007). There are currently no legislative limits for the mycotoxin content of beers. The Scientific Committee on Food has set a TDI for fusarium toxins, for ZEA content at 0.25 μg/kg body weight per day. Is the dose that a consumer can consume daily without a significant increase in harm to his health. The amount of food consumed and the weight of the average person are also important factors (Martiník et al., 2023, Ksieniewicz-Woźniak et al., 2019). In the case of the occurrence of ZEA in beer, it is important to note that the mycotoxin ZEA usually passes very rarely into beer because it is metabolised by yeasts during the brewing process, mainly to β-zearalenol. However, during the processing of malt infected with moulds, changes in the composition of the intermediate products of beer production can occur, which can negatively affect the brewing, flavour, textural and foaming properties of the beer (Wolf-Hall, 2007). Minimum levels of ZEA, safe for consumption, were found in the beer samples analysed (Figure 3). In the case of Bojos beer, the treatment method had no effect on the variability of the samples, no statistically significant difference was found between the samples ($P > 0.05$). On the other hand, significant statistical differences ($P < 0.05$) were observed for the Francin beer sam-





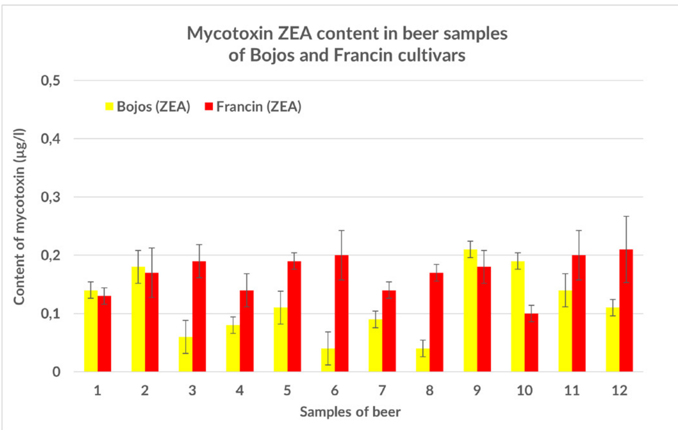
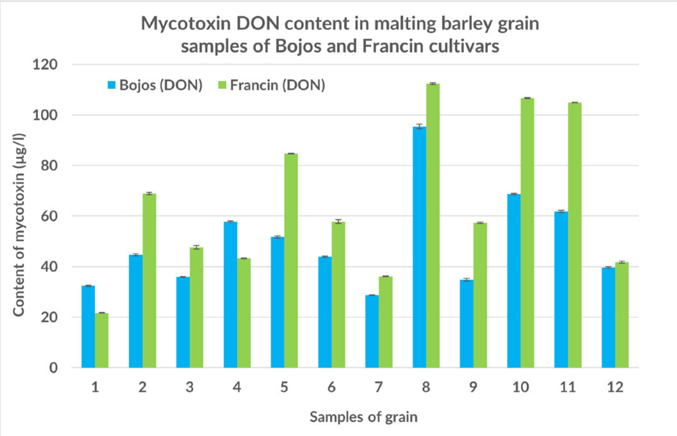
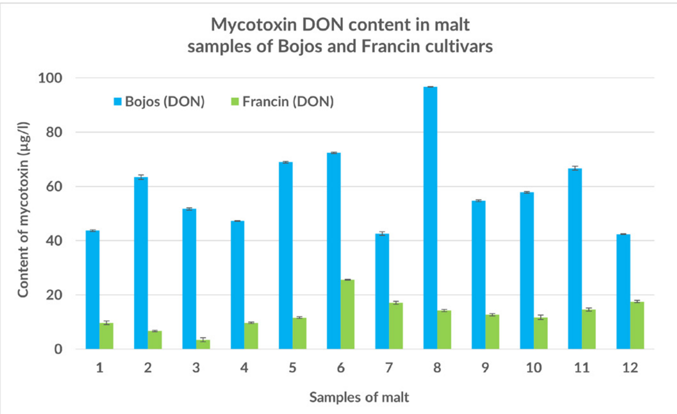


Figure 1 *Comparison of the zearalenone (ZEA) content in variants of grain samples of cultivars Bojos and Francin*

Figure 2 *Comparison of the zearalenone (ZEA) content in variants of malt samples of cultivars Bojos and Francin*

Figure 3 *Comparison of the zearalenone (ZEA) content in variants of beer samples of cultivars Bojos and Francin* ples. In the case of Francin, an association with the level of nitrogen fertilization was observed. The ZEA content of the beer increased with increasing nitrogen dose. For the variety Bojos this trend was not analysed for the individual sample variants. Nevertheless, mycotoxins are considered to be a problem in the brewing industry and therefore need to be monitored in beer and raw materials for beer production (Martiník et al., 2023, Oliveira et al., 2012).





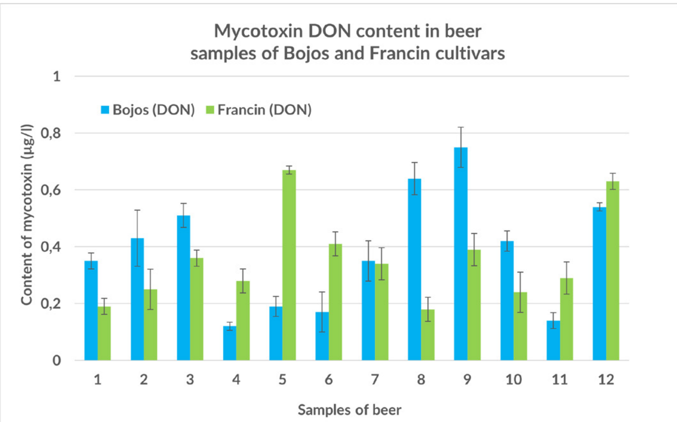


Figure 4 *Comparison of the deoxynivalenol (DON) content in variants of grain samples of cultivars Bojos and Francin*

Figure 5 *Comparison of the deoxynivalenol (DON) content in variants of malt samples of cultivars Bojos and Francin*

Figure 6 *Comparison of the deoxynivalenol (DON) content in variants of beer samples of cultivars Bojos and Francin*

3.2 Determination of the mycotoxin deoxynivalenol (DON) in grain, malt and beer

The mycotoxin DON in cereals is highly fluctuating from year to year. In particular, it depends on the climatic conditions in the area where it is being planted, the type of pre-crop used and the resistance of the cultivar (Malíř et al., 2023). DON is considered one of the least toxic trichothecenes. DON is stable to factory processing, e.g. heating, milling and manufacturing, and therefore easily gets into the food chain (Malíř et al., 2023). Commission Regulation (EU) 2023/915 also adjusts the maximum content of DON in unprocessed cereal grains to 1000 µg/kg. Statistically significant differences ($P < 0.05$) were found between grain samples of both Bojos and Francin cultivars. When comparing the incidence of DON between cultivars, the measured values did not differ much. Nevertheless, the measured DON values were higher in the grain of the Francin cultivar, which may indicate that it may be more susceptible more susceptible to contamination by this mycotoxin. The highest grain DON value in both Bojos and Francin was again found in the variant of sample 8 – nitrogen fertilization rate of 80 kg/ha, with fungicide application, without artificial infection (Figure 4), as well as in the occurrence of ZEA in grain. None of the cereal samples monitored exceeded by far the legal limits. Higher DON content in Francin grain was observed in the variants of the samples with nitrogen fertilization of 80 kg/ha. The previous harvest, in our case sugar beet, is also an important factor for the presence of mycotoxins. *Fusarium* microspores are ubiquitous and mere contact of the harvested crop with the soil at harvest can be risky (Wolf-Hall, 2007).

An interesting finding was that in the case of comparison of DON content in grain, the higher DON content in grain was in the Francin cultivar. However, when comparing the DON content in malt, significantly higher values were measured for the Bojos cultivar. Wolf-Hall (2007) reports that mycotoxin levels usually decrease during malting itself but may increase during germination. Further mycotoxin production can occur during drying. High temperatures limit mould growth, but for some moulds the increase in temperature during the early stages of the fermentation process may still encourage the production of mycotoxins in the malt. As stated above, the highest DON level in grain of the Bojos cultivar was in sample 8 (Figure 4). Almost the same mycotoxin content was subsequently transferred from the grain sample to the malt (Figure 5). Samples of Bojos malt cultivar were statistically different ($P < 0.05$). However, there were no statistically significant differences ($P > 0.05$) between the results of DON content in malt samples of the cultivar Francin. Bauer et al. (2016), also confirmed that the mycotoxin content already present in raw grain may increase due to the promoted fungal growth in the malting process. These findings are also confirmed by Lancová et al. (2008). In their study, they report that soaking barley grains apparently reduced the levels of *Fusarium* mycotoxins, but during germination their gradual accumulation occurred.

The maximum limit for DON in beer is not set by European legislation, but the European Food Safety Authority (EFSA) has set the TDI for DON at 1 µg/l. None of the samples, however, exceeded the TDI limit of 1 µg/l. The Bojos beer samples were not statistically different in DON content ($P > 0.05$). The higher DON content of the malt sample variant 8 was also reflected in the beer Bojos, sample variant 8 (Figure 6), made from it. Arrúa et al. (2019) describe that DON in the brewing process is transferred from barley through the malt to the final beer product and its concentration increases during the production process. The highest value for the Bojos beer was found for sample variant 9 (0.75 µg/l), yet it would be safe for consumers to consume. The increase in DON content during malt and beer production may be up to an order of magnitude higher and may be related to the activity of hydrolytic enzymes that contribute to the release of mycotoxins from their binding to polysaccharides (starch). Their concentration increases with increasing fermentation time, i.e. with alcohol content (Ksieniewicz-Woźniak et al., 2019; Wolf-Hall, 2007). The results for the samples of the Francin beer variety were statistically different ($P < 0.05$). The highest level of DON in beer was measured in beer Francin, in sample 5 (0.67 µg/l). Significantly higher value was also analysed for the variant of samples 12, compared to the other samples. Samples 5 and 12 were both contaminated with *F. culmorum*. However, none of the beer samples monitored exceeded the TDI limit of 1 µg/l.

4 Conclusion

From our results, it is clear that statistically significant differences ($P < 0.05$) were observed when monitoring ZEA and DON content in grain samples of both Bojos and Francin cultivars. A lower incidence of ZEA in grain was observed in the Francin cultivar. However, the measured values indicate that the Bojos cultivar shows a higher resistance to the presence of DON in grain. Different levels of nitrogen fertilisation were also monitored in the experiment. However, the hypothesis that higher levels of nitrogen fertilisation increase the susceptibility of the crop to fungal attack and subsequent mycotoxin formation was not confirmed. Nevertheless, doses of 80 and 110 kg/ha may have contributed to the increasing mycotoxin levels in grain. From a biocenotic standpoint, plants are typically colonized by the indigenous microflora of their growth environment, so the effects of a controlled inoculation may not be observable. Variation in ZEA levels among the individual treatments may also be due, among other factors, to the use of *Fusarium culmorum* for inoculation, which primarily promotes DON production; by contrast, as reported in the literature (Park et al., 2016), *Fusarium graminearum* is a major producer of ZEA. In the competitive context of the resident microflora, the impact of controlled contamination with the *F. culmorum* strain may therefore go undetected, as confirmed in this case. Moreover, the microbial infection pressure at the experimental site may have exceeded that imposed experimentally by the *F. culmorum* strain—conditions that are generally very difficult to disentangle (Nahle et al., 2021).

Mycotoxins cannot be completely removed from already contaminated barley grain. The quality of the raw materials therefore plays a decisive role in the organoleptic characteristics of the beer and it is recommended to pay special attention to their hygienic quality. To ensure their quality, it is necessary to check them regularly and not to use contaminated grain for malting and brewing. The malting process may in turn contribute to an increase in the mycotoxin content of the final product. Malting conditions generally encourage the production of mycotoxins. Optimising them could therefore be an effective measure to suppress mould growth, reduce mycotoxin production and maintain the overall quality of malt and beer. Probably the higher temperature chosen during kilning could degrade mycotoxin production. It is also known from the available literature that the removal of the germ, where the mycotoxin content may accumulate most, would be advisable. Foreign authors have reported that some yeast strains can also bind dangerous mycotoxins that are transferred to the final product. These recommendations could therefore be the subject of further investigation. The results for the ZEA content of malt in both Bojos and Francin were not statistically different ($P > 0.05$). When monitoring the DON content of the Bojos malt samples, significant differences were found ($P < 0.05$). On the other hand, for the Francin cultivar, the DON content values were statistically comparable ($P > 0.05$).

Beer does not have to be contaminated just by using unsuitable ingredients. It is also worth noting the possible source of secondary contaminants in the finished product. These contaminants enter the beer during bottling, canning or kegging. It is therefore recommended proper cleaning and sanitation of the brewing equipment to avoid contamination of the beer. A 0.5% peracetic acid solution was used in the experiment. Furthermore, it would be advisable to introduce monitoring systems in the production chain to guarantee the quality and safety of the product. The results of the Bojos beer samples were not statistically different in ZEA and DON content ($P > 0.05$). In the case of the Francin beer samples, statistically significant differences were observed in ZEA and DON content ($P < 0.05$).

Currently, the most common detection method is high-performance liquid chromatography (HPLC) for the analysis of mycotoxins, which allows the simultaneous determination of compounds at relatively low concentrations. Although mycotoxins in the raw materials for beer production and in the finished product itself do not pose a significant health risk, their presence must be monitored to protect the health of consumers. Various regulatory limits for the presence of certain mycotoxins are in place worldwide for cereals or specific derived products, but for most mycotoxins in beers there is no technical regulation on maximum tolerable limits, only for raw materials. For beer only a maximum tolerable daily/weekly intake is established. When monitoring the presence of the mycotoxins ZEA and DON, we did not find any significant values in grain and malt exceeding the limit set by the legislation. The impact of mycotoxins on human health is still underestimated, although the prevalence of fungi and their mycotoxins are a hot topic and determining the risk associated with their intake in the diet of the human population is an evolving process.

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6 References

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