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The fate of deoxynivalenol and its derivatives in spring wheat whole-grain flour during storage

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Abstract

A study was conducted at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry to explore the quantitative changes in type B trichothecenes: deoxynivalenol (DON), 3-acelyt-deoxynivalenl (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), in spring wheat whole-grain flour as influenced by storage period and temperature. Samples with different concentrations of DON and its metabolites were selected and stored in two controlled climate chambers at different temperatures (18°C and 28°C) at the same ambient air humidity (~80%). The initial concentration ranged from 1246 to 4581 μg kg^{-1} for DON, from 440 to 820 μg kg^{-1} for 3-ADON and from 88 to 141 μg kg^{-1} for 15-ADON. The samples were analysed before the storage experiment and then after 4 and 8 weeks of storage. The findings of the DON retention showed no significant differences between the storage periods and temperatures; however, it was found that over time the concentrations of DON in the samples decreased. There were found significant differences ($P < 0.05$) in 3-ADON concentrations between storage periods and temperatures. Over time, 3-ADON concentrations decreased in the samples. Statistically significant increases in 15-ADON concentrations were identified over this period. Within 60 days of storage, the concentrations of DON decreased by 16% and 33%, 3-ADON – by 60% and 100%, and those of 15-ADON increased by 63% and 96% compared to the initial levels, depending on the combination of the experimental factors.

The results of this study led to the conclusion that storage of spring wheat flour for several months at 80% relative humidity, at 18°C and 28°C temperatures did not have significant influence on the retention level of DON. However, the levels of DON derivative 3-ADON significantly decreased and the concentrations of 15-ADON significantly (almost twice) increased during flour storage and aging.

Key words: flour, high-performance liquid chromatography, humidity, temperature, type B trichothecenes.

Introduction

It is known that agricultural commodities and particularly cereals are prone to fungal infection during growth, harvest, transport and storage. As a result, agricultural commodities are often contaminated with mycotoxins leading to acute and chronic health exposure. Such exposure may result in acute visible symptoms but also can result in long-term latent health damage. Mycotoxins are mainly produced by the species of *Aspergillus*, *Penicillium*, *Alternaria*, *Claviceps* and *Fusarium* (Udovicki et al., 2018). Among them, the most common mycotoxin associated with wheat is DON, which is also referred to as vomitoxin (a type B trichothecene) (Sobrova et al., 2010). DON is produced mainly by *Fusarium* species, such as *F. graminearum* and *F. culmorum*. Both *F. graminearum* and *F. culmorum* are listed as pathogens of wheat that cause Fusarium head blight (FHB), which is also identified as scab. The disease leads to a reduced yield, lower grade and end-use quality of wheat grains. Mycotoxins are secondary metabolites produced mainly by moulds under certain conditions. The most common toxigenic fungi in Europe are *Fusarium* species, which produce trichotecene-class mycotoxins, such as DON and its derivatives 3-ADON and 15-ADON. DON, a toxic secondary metabolite produced by several *Fusarium* species in the grains of various cereals, is one of the most frequently occurring type B trichothecenes worldwide, which causes many adverse health effects to humans and animals (Warth et al., 2012).

It has been reported that 25–50% of harvested crops in the world are contaminated with mycotoxins annually (Ricciardi et al., 2013). Due to plant responses, matrix effects and reactions occurring during food processing, modified and other configurations of DON can be formed in wheat and wheat-based products (Berthiller et al., 2013; Rychlik et al., 2014). The main factors that favour fungal growth and mycotoxin biosynthesis in stored grain are high grain moisture (16–30%), warm grain temperature (25–32°C) and high air relative humidity (80–100%) (Shanahan et al., 2003).

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The optimum temperature for DON production ranges between 26–30°C (Milani, 2013). It has been documented that a temperature rise of 2–3°C may indicate mould growth or insect infestation (Neme, Mohammed, 2017).

Spring wheat was more heavily contaminated with DON than winter cereals in Lithuania. Moreover, DON concentrations in spring wheat grain were also higher than those in winter wheat grain (Mankevičienė et al., 2014). Also it was found that *F. graminearum* in spring wheat grain had stronger correlation ($r = 0.783$, $p < 0.01$) with deoxynivalenol (DON) content (Supronienė et al., 2016). In Lithuania, in spring wheat grain samples 15-ADON co-occurred with DON and 3-ADON (Janaviciene et al., 2018). From the viewpoint of food safety, it is essential to be aware of which trichothecene predominates in grain samples, as sample toxicity depends on this. It is thought that 15-ADON is more toxic than DON and 3-ADON (Pinton et al., 2012).

Researchers suggest that DON can be degraded or detoxified into various derivatives by acetylation (Berthiller et al., 2005; Karlovsky, 2011; Warth et al., 2012). High moisture (>70%) and temperature (>25°C) conditions are favourable for *Fusarium* development in the field. *F. graminearum* grows well at 20–30°C (Brennan et al., 2003; 2005; Doohan et al., 2003; Xu et al., 2007) with optimal temperatures ranging between 25–29°C (Kang, Buchenauer, 2002; Xu, 2003). However, apart from being seed-borne pathogens, they may also grow on stored products (Khanzada et al., 2002; Gonçalves et al., 2019). There are many studies addressing the fate of mycotoxins during storage. Mycotoxin retention levels in stored grain are closely related to the species and state of grain as well as the storage environment. However, there is a paucity of published data on the fate of 3-ADON and 15-ADON during grain storage. These derivatives are of particular importance. Storage under controlled conditions, including packaging practices, temperature control, ventilation efficiency and proper air humidity, will reduce fungal development and mycotoxin accumulation (Shanakht et al., 2014).

Therefore, proper exposure and risk assessment models that will support decision-making should not only rely on the occurrence and quantification of DON but also on the modified forms of DON (acetyl derivatives and conjugated forms). Surely, the consideration of modified forms of DON in DON exposure and risk assessment models must be supported by previous studies on their relevance for public and animal health (Khaneghah et al., 2018).

The presence of these modified forms of DON (i.e. masked mycotoxins) has been raising significant concerns regarding the safety of contaminated products. Also, masked mycotoxins may represent analytical challenges, as the native mycotoxin may not be detected, while the modified mycotoxin still retains the toxicological effects (Galaverna et al., 2009). To protect consumer health, harmonized regulations for DON in cereals and derived products, including official protocols for sampling and analysis, were implemented within the European Union (EU). The current EU maximum permitted levels for DON have been fixed at 1250 μg kg^{-1} for unprocessed cereals, 1750 μg kg^{-1} for unprocessed durum wheat and oats, 750 μg kg^{-1} for foodstuffs intended for direct human consumption and dry pasta, 500 μg kg^{-1} for breakfast cereals and snacks, and 200 μg kg^{-1} for cereal-based infant food (European Commission, 2006).

Currently, there are not regulatory levels for the most frequently occurring modified forms of DON, i.e. 3-ADON and 15-ADON in cereals and cereal-based foods. However, the FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) considered that the acetylated derivatives might contribute to exposure to DON. Based on the available toxicity data, the Committee considered the toxicity of the acetylated derivatives equal to that of DON (JECFA, 2010). Following a request of the European Commission, the risk toward these acetylated and modified forms of DON (3-ADON, 15-ADON and DON-3-Glc) has been assessed for human and animals health. 3-ADON and 15-ADON elucidated in the intestines suggest the same biological activity as described for DON, and that the estimated chronic dietary exposures indicated potential health concerns (EFSA, 2017). Therefore, surveillance and control of DON and its acetyl derivatives in foods and feeds are of great importance for producers, researchers and regulatory authorities to protect consumer health and to reduce economic losses (Liu et al., 2016). To retain better wheat quality, it should be stored below 25°C temperature at 60% relative humidity (Abdullah et al., 2019).

The aim of this study was to extend the knowledge on the fate of DON, 3-ADON and 15-ADON in spring wheat whole-grain flour stored in different environments during a two-month storage period, and establish the influence of grain state, storage temperature on the fate of DON, 3-ADON and 15-ADON during storage.

Since mycotoxin contamination of food and feed raw materials is hardly avoidable, the data on mycotoxin occurrence are of great importance for food safety. However, the data concerning the distribution of mycotoxins in stored cereals and cereal-based products are rather limited. There is little information on the prevalence and fate of 3-ADON and 15-ADON during flour storage.

Materials and methods

Grain and flour samples. Spring wheat grains naturally infected with *Fusarium* fungi were collected from commercial fields of Lithuania (crop years 2013 and 2014) and analysed in 2018. A total of 103 one-kg samples of spring wheat grain were collected and analysed for the co-occurrence of type B trichothecenes (DON, 3-ADON and 15-ADON). The grain samples were stored at −18°C temperature until analyses. Analyses of mycotoxins were done at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry.

Seven grain samples with high concentrations of DON, 3-ADON and 15-ADON (four replications) were chosen for the storage experiment. The initial concentrations of DON, 3-ADON and 15-ADON in the samples of wheat grain naturally infected with *Fusarium* fungi were determined by a high-performance liquid chromatography (HPLC) assay. The initial concentration ranged from 1246 to 4581 μg kg^{-1} for DON, from 440 to 820 μg kg^{-1} for 3-ADON and from 88 to 141 μg kg^{-1} for 15-ADON. The moisture content of the grain prior to milling was ~10% ±0.5. Before the storage experiment, flour was produced from the spring wheat grain samples. The flour sample with the highest concentration of mycotoxins was chosen for comparison of the experimental effects and laboratory measurements.

Storage conditions. The experiment was designed as one-factorial arrangement. The studied factor was storage environments (18/28°C temperature and ~80% relative humidity). Wheat grain flour samples were put on glass plates, placed in a controlled climate chamber (Binder, Germany) and stored for 8 weeks in two storage environments. The experiment continued from March to May, 2018. The levels of DON, 3-ADON and 15-ADON in all flour samples were analysed by HPLC-UV before the storage experiment and after 4 and 8 weeks of storage.

Chemicals and reagents. HPLC grade acetonitrile (C_2H_3N), methanol (CH_3OH) and polyethylene glycol (PEG) 8000 were obtained from Merck (Germany). Certified mycotoxin standards for DON, 3-ADON and 15-ADON were purchased from LGC standards (Germany). Wheat reference material was purchased from Trilogy Analytical Laboratory (USA). Deionized water with resistivity of 18.2 MΩ was generated by a Milli-Q plus system (Millipore, USA). For the sample clean-up step, an immunoaffinity column NeoColumn for deoxynivalenol (DON) (NEOGEN Europe Ltd., Germany) was used according to the manufacturer's procedures.

Sample preparation. All grain samples were ground in an Ultra Centrifugal Mill ZM 200 (Retsch, Germany) with 0.8 mm sieve. The ground spring wheat samples were analysed according to the method previously validated for the determination of DON in cereals with some modifications (Kotal, Radova, 2002). For extraction, 10 g of ground sample was placed into 100 ml glass bottle and then 40 ml deionized water and 2 g PEG were added. The mixture was stirred for 1 min. The extract was filtered through a fluted filter and then through a microfiber filter. One ml of the final extract, corresponding to 0.25 g of the original material, was placed into the DON test column. For column washing 10 ml of redistilled water was used. The elution of DON was done by 1 ml CH_3OH. The elution solvent was evaporated to dryness (at 50°C) and dissolved in 1000 µl mobile phase (H_2O:C_2H_3N 90:10). The extract was ready in a chromatographic system (Kotal, Radova, 2002).

High-performance liquid chromatography (HPLC) analysis. In the study, quantitative and qualitative analyses of mycotoxins were performed using high performance liquid chromatography. The identification of mycotoxins was performed on a HPLC system (Shimadzu, Japan), consisting of a LC-20 AT pump equipped with a FCV-10AL quaternary valve, a SIL-20A autosampler, a DCU-20A5 degasser, a CTO-20A column oven, a UV detector (Shimadzu); wavelength of 218 nm, equipped with YMC-Pack Pro C18, 3 µm (4.0 × 150 mm) column. Data collection and evaluation were performed by using an operating system LCsolution LC/GC, 5.42 (Shimadzu). Chromatographic peaks were identified by overlaps between analytes and standard compound retention times. HPLC conditions of DON, 3-ADON and 15-ADON are shown in Table 1.

Table 1. High-performance liquid chromatography (HPLC) conditions of DON, 3-ADON and 15-ADON

Parameters	DON	3-ADON	15-ADON
HPLC column	YMC-Pack Pro C18 3 µm, 4.0 × 150 mm		
Flow rate ml min^{-1}	0.8		
Detector, wavelength	UV 218–220 nm		
Column temperature °C	40°C	30°C	30°C
Mobile faze	H_2O:C_2H_3N:CH_3OH, 92:4:4 v/v/v	H_2O:C_2H_3N 90:10 v/v	H_2O:C_2H_3N 90:10 v/v
Injection volume µl	100	50	50

Gradient elution conditions for DON, 3-ADON and 15-ADON are shown in Table 2.

The mobile phases A and B for DON were 100% C_2H_3N and 100% CH_3OH, respectively, and retention time was 35 minutes. The mobile phases A and B for 3-ADON were 100% C_2H_3N and 90:10% H_2O:C_2H_3N, respectively, and retention time was 20 minutes. The mobile phases A and B for 15-ADON were 100% C_2H_3N and 100% H_2O, respectively, and retention time was 20 minutes.

Table 2. Gradient elution conditions for DON, 3-ADON and 15-ADON

Time, minutes	Mobile phase A %	Mobile phase B %
DON		
0–15	100	0
15–25	0	100
25–35	100	0
3-ADON		
0–2	0	100
2–5	0→20	100→80
5–13	20	80
13–15	20→0	80→100
15–20	0	100
15-ADON		
0–2	20	80
2–5	20→30	80→70
5–13	30	70
13–15	30→20	70→80
15–20	20	80

Method validation. HPLC with UV detection is generally not applicable to most trichothecenes exhibiting weak UV absorption, whereas it is routinely used for quantitative analysis of DON, and its acetylated derivatives in cereals with good accuracy and precision (MacDonald et al., 2005; Stroka et al., 2006). The established HPLC methods were assessed for linearity, recovery, precision, limit of detection and limit of quantification (ICH, 2005). The five-point calibration curve was made in the concentration ranges 2–40 µg ml^{-1} for DON. A five-point calibration curve was made in the concentration ranges 1–25 µg ml^{-1} for 3-ADON, and a six-point calibration curve was made for 15-ADON in the concentration ranges 1–50 µg ml^{-1}. Each standard solution was injected into chromatographic column three times and the working range was established.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated on the basis of signal – to noise – ratio and were: S/N = 3 for LOD and S/N = 10 for LOQ. The repeatability was determined for two 1000 and 2000 µg kg^{-1} concentrations, and each group

of fortified samples was analysed in one day. Calculations were performed of the recoveries (%), relative standard deviation (RSD, %), LOD, LOQ and also the correlation coefficients (*R*), and linear ranges were determined for each mycotoxin analysed (Table 2).

Statistical analysis. Means of mycotoxin concentrations and the standard errors of the means at the beginning and at the end of storage were calculated. We attempted to describe the changes (increases or decreases) in mycotoxin concentrations during storage numerically. The average values and standard deviation of the results were calculated using the software *Microsoft Office Excel 2010* (USA). The significance of the data was evaluated using the statistical data processing program *SAS Enterprise Guide*, version 7.1 (SAS Institute Inc., USA) and different statistical packages. One-way analysis of variance (*ANOVA*) statistical package was used to evaluate data scatter and to determine significant differences between the means. The essential differences between the two samples were compared using the Duncan's criterion. Zero was used instead of not detected to calculate significant differences. The differences were statistically significant with a confidence level of $p < 0.05$.

Results

Validation results. According to the linear regression analysis, the calibration curves for DON were linear from 2 to 40 µg ml^{-1}, for 3-ADON – from 1 to 25 µg ml^{-1} and for 15-ADON – from 1 to 50 µg ml^{-1}, which showed the correlation coefficient *R* of 0.9998 to DON, 0.9994 to 3-ADON and 0.9998 to 15-ADON. Retention times for DON, 3-ADON and 15-ADON were 10.5, 9.8 and 5.8 min, respectively. The LODs – 3 s/n (s/n – signal-to-noise ratio) of DON, 3-ADON and 15-ADON were 13, 19 and 19 µg kg^{-1}, respectively, and the LOQs – 10 s/n (s/n) were 46, 64 and 63 µg kg^{-1}, respectively. The mean recoveries were 99 ± 3%, 100 ± 6% and 102 ± 7% for DON, 3-ADON and 15-ADON, respectively. These results suggest that the chosen analytical method exhibited good accuracy and precision for the detection of DON, 3-ADON and 15-ADON in spring wheat grain flour samples (Table 3).

Table 3. Validation parameters of DON, 3-ADON and 15-ADON

Validation parameters	Results		
Mycotoxin	DON	3-ADON	15-ADON
Limit of detection (LOD) µg kg^{-1}	13	19	19
Limit of quantification (LOQ) µg kg^{-1}	46	64	63
Correlation coefficient (*R*)	0.99	0.99	0.99
Mean recovery %	98.85	100.04	102.14
Repeatability RSD%	3.54	6.08	7.11

RSD – relative standard deviation

Quantification of mycotoxins in spring wheat grain samples. The validated methods were applied for all 103 spring wheat grain samples to determine DON, 3-ADON and 15-ADON. The results showed DON to be present in 97%, 3-ADON in 74% and 15-ADON in 67% of the spring wheat grain samples analysed. The levels of DON and its derivatives in positive samples are shown in Table 4.

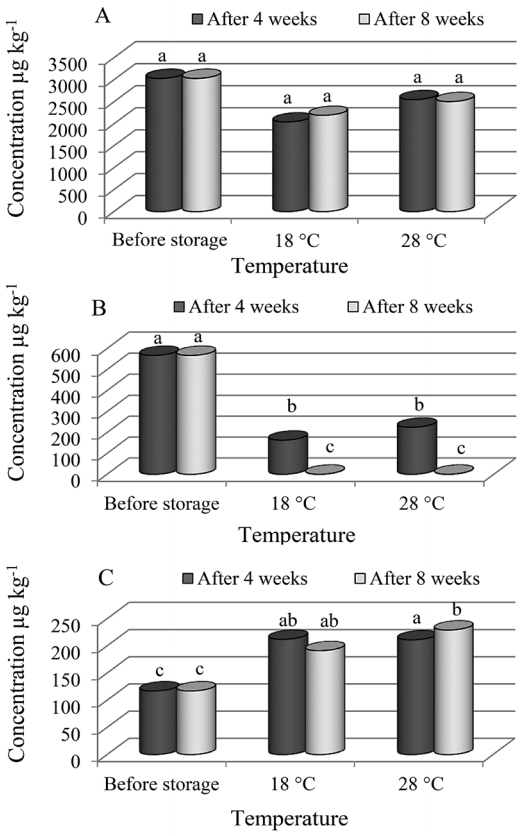
Table 4. The levels of DON and its derivatives in positive samples

Mycotoxin	Positive samples %	Range µg kg^{-1}	Mean of positive samples µg kg^{-1}	Mean value µg kg^{-1}
DON	97	<LOD–6804	537	495
3-ADON	74	<LOD–1149	257	160
15-ADON	67	<LOD–1318	262	93

<LOD – below limit of detection

Changes of DON, 3-ADON and 15-ADON concentrations during storage in spring wheat grain flour. The flour was made from the selected 7 spring wheat grain samples shortly before the storage experiment. The mean concentrations of DON and its derivatives before storage and at different storage periods for each specific combination of the storage factors are presented in Figure. This study was focused for the first time on the dynamics of 3-ADON and 15-ADON together with DON during wheat whole-grain flour storage.

The results of the study showed that there were no statistically significant differences in DON concentrations between the storage periods and different temperatures; however, Figure A shows that DON concentrations in the samples decreased over time. The mean initial concentration of DON was 3032 ± 489 µg kg^{-1}. A greater decrease in DON concentrations was



Note. Different letters a–c to show statistically significant differences between variables.

Figure. The mean concentration of DON (A), 3-ADON (B) and 15-ADON (C) after 4 and 8 weeks of storage

observed at 18°C temperature, where the mean DON concentration dropped to 2043 ± 61 μg kg^{-1} after 4 weeks and to 2550 ± 119 μg kg^{-1} after 8 weeks of storage. The degradation of DON occurred most rapidly when the flour was stored for 4 weeks at 18°C temperature (33%). The average retention level of DON in wheat grain flour samples after all storage period was ~76.5%.

Within 8 weeks of storage, 3-ADON concentrations decreased in the samples. Significant differences in 3-ADON concentrations were found between the storage periods and temperatures (Fig. B). The mean initial concentration of 3-ADON was 567 ± 119 μg kg^{-1}. A greater decrease in 3-ADON concentrations was observed at 18°C temperature, where the mean 3-ADON concentration dropped to 163 ± 24 μg kg^{-1} after 4 weeks. Meanwhile, the average reduced level of 3-ADON in the samples stored at 18°C temperature (14.5% retention level) was higher than that of samples stored at 28°C temperature (20% retention level). In the samples stored at 18°C and 28°C temperatures, 3-ADON concentrations were undetectable after 8 weeks of storage. The average retention level of 3-ADON in wheat grain flour samples after 8 weeks of storage was 17.25%.

The concentrations of DON and 3-ADON decreased during storage, but this was not the case with 15-ADON (Fig. C). The mean initial concentration of 15-ADON was 117 ± 19 μg kg^{-1}. After 8 weeks of storage, 15-ADON concentrations in the samples were almost twice as high as those before storage. Significant differences in 15-ADON concentrations were determined in the flour stored for 8 weeks at 28°C temperature. The concentrations increased to 96%. No significant differences in 15-ADON concentrations were found between the storage periods in the flours stored at 18°C temperature.

Within 60 days of storage, the concentrations of DON decreased by 16% and 33%, 3-ADON decreased by 60% and 100% and those of 15-ADON increased by 63% and 96% compared to the initial levels depending on the combination of the experimental factors.

Within 4 and 8 weeks, the concentrations of 15-ADON statistically significantly increased, and the average retention level in wheat grain flour samples after storage at 18°C temperature was 72% compared to the concentration before storage. However, significant differences in 15-ADON concentrations were found between the storage periods in the flours stored at 28°C temperature. The average retention level of 15-ADON in wheat grain flour samples after storage was 80%.

Discussion

To garner more knowledge about the potential health risk associated with these frequently occurring mycotoxins, their fate during storage should be thoroughly examined. The JECFA (2010) emphasized the significance of acetylated derivatives of DON (3-ADON and 15-ADON) in the total DON-induced toxicity. Recently, European Food Safety Authority (EFSA) reported on the risks to human and animal health related to the presence of DON and its acetylated and modified forms in food and feed (EFSA, 2017). The occurrence of DON in wheat grain flour is an issue of global concern and a relevant topic for regulatory agencies (Lanza et al., 2019). On the other hand, although mycotoxins, such as DON and its derivatives, have high thermal stability, the effects of different thermal treatments on mycotoxins vary greatly (Bretz et al., 2006).

In our study, we focused on the dynamics of DON, 3-ADON and 15-ADON during spring wheat grain flour storage to fully understand retention of DON and its derivatives. Zhang et al. (2016) showed that DON concentrations in wheat grain decreased more than 40% within 30–90 days of storage. Contrary to our results, Zhang et al. (2016) reported that DON concentration increased in wheat grain flour. In our study, DON concentrations in wheat grain flour decreased 23.5%. While DON concentration in all samples during the whole storage period showed a generally continuous increase, which reached more than 70% after storage, and the results were completely contrary to those of wheat grain (Zhang et al., 2016). On the contrary, the levels of DON can decrease during spring wheat grain flour storage. This finding agrees with Aleš et al. (2010), who reported that levels of DON can decrease during organic whole-grain wheat flour storage. Mankevičienė et al. (2019) showed that DON concentrations after 28 days of storage at 20°C and 28°C temperatures were 34% higher ($p < 0.05$) compared with those before storage.

Palacios et al. (2017) showed that acetylated derivatives (3-ADON and 15-ADON) of DON were found in 49% of the wheat grain samples. Mycotoxins can co-occur naturally in grain. Janaviciene et al. (2018) identified 5 combinations of mycotoxins in spring wheat grain. Samples contaminated with DON alone accounted for 17.5%, DON + 3ADON for 11.7%, DON + 15ADON for 5.8% and DON + 3ADON + 15ADON for 61.2% of the total samples analysed. Only one sample was contaminated with 3-ADON alone (1%), and 15-ADON alone was not detected in the tested samples. No toxins were detected in 2% of the spring wheat samples. However, Zhang et al. (2019) showed that DON alone made up 62.9% in whole-grain wheat flour, DON + 3-ADON and DON + 15-ADON accounted for 5.7%. The combination of three mycotoxins (DON + 3-ADON + 15-ADON) in whole-grain wheat flour made up 8.6%.

Only a few research papers have reported the natural incidence of the modified forms of DON in wheat grain flour. To our knowledge, little information is available on the stability of 3-ADON and 15-ADON during storage. Although DON and it derivatives are similar in chemical structure, studies on the conversion profile of these compounds were scarce, especially during flour storage. Knowledge on the fate of conjugated mycotoxins during flour storage is becoming increasingly urgent because of the rising concern about the risk assessment for mycotoxins. In our study, chromatographic analysis conducted after 8 weeks of storage showed that 3-ADON was not detected in the samples stored either at 18°C or 28°C temperatures. This suggests that storage of flour for longer than 8 weeks' period can significantly reduce 3-ADON contents, regardless of the environmental conditions.

Flour types, storage conditions, microbial activity and many other factors affect complex processes during flour storage, so the integrative effects lead to

different results (Cenkowski et al., 2000; Zhang et al., 2016). At harvest and storage of cereals, spore containing material and humidity combined with temperature are the key factors affecting trichothecene formation. Minimising or avoiding spore containing materials, grain cleaning at harvest and drying at low temperatures will allow storage of cereals for more than 12 months without increasing trichothecene levels (Schrödter, 2004). Storage conditions play an important role in mycotoxin control, since they will influence overall fungal development. In general, high humidity and temperature can favour fungal growth and promote mycotoxin production (Hina et al., 2014). Harvesting time and various storage systems exert a major impact on wheat, but high temperature and humidity during storage are detrimental for wheat grain health, quality, and germination (Abdullah et al., 2019).

The lower the DON concentration, including DON derivatives in wheat grain, the lower the risk that the legally binding threshold is exceeded. Nevertheless, food safety consideration requires a wide-ranging discussion on the need to update legislation concerning the threshold in view of the fact that DON derivatives commonly accompany DON itself (Bryła et al., 2018).

Conclusions

1. Based on the results of this experiment, it was concluded that storage of spring wheat whole-grain flours for several months under conditions of 18/28°C temperature, ~10% flour moisture content and 80% relative humidity does not significantly influence the retention level of trichothecene deoxynivalenol (DON).

2. Storage of spring wheat grain flours for over 8 weeks at both 18°C and 28°C temperatures at 80% relative humidity resulted in a significant decrease in 3-ADON concentrations and a significant increase in 15-ADON concentrations ($p < 0.05$).

3. Environmental factors, such as ambient temperature and humidity can significantly influence mycotoxin production in stored grain and flour. Therefore, further research in this field would facilitate control of the incidence of these toxins in food products and would contribute to food quality and safety.

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Deoksinivalenolio ir jo darinių kitimas vasarinių kviečių viso grūdo miltuose laikymo metu

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Santrauka

Siekiant ištirti kiekybinius B tipo trichotecenų: deoksinivalenolio (DON), 3-acetil-deoksinivalenolio (3-ADON) ir 15-acetildeoksinivalenolio (15-ADON), koncentracijų pokyčius vasarinių kviečių viso grūdo miltuose, priklausomai nuo laikymo trukmės ir sąlygų, Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institute 2018 m. buvo atliktas tyrimas. Mėginiai su įvairiomis DON ir jo metabolitų koncentracijomis buvo atrinkti ir laikomi dviejose kontroliuojamo klimato kamerose su skirtingomis temperatūromis (18° ir 28° C) ir vienoda aplinkos oro drėgme (~80 %). Pradinė DON koncentracija svyravo nuo 1246 iki 4581 µg kg^{-1}, 3-ADON – nuo 440 iki 820 µg kg^{-1}, 15-ADON – nuo 88 iki 141 µg kg^{-1}. Mėginiai buvo analizuoti prieš laikymo eksperimentą ir po 4 bei 8 saugojimo savaičių. DON neparodė reikšmingų skirtumų tarp laikymo trukmės ir temperatūrų, tačiau buvo nustatyta, kad laikymo metu DON koncentracija mėginiuose sumažėjo. 3-ADON koncentracijose tarp laikymo trukmės ir temperatūrų buvo esminių skirtumų ($P < 0,05$). Laikymo metu 3-ADON koncentracija mėginiuose sumažėjo. Per tyrimo laikotarpį buvo nustatytas esminis ($P < 0,05$) 15-ADON koncentracijos padidėjimas. Nustatyta, kad per 60 dienų laikymo laikotarpį DON koncentracija sumažėjo nuo 16 iki 33 %, 3-ADON – nuo 60 iki 100 %, o 15-ADON koncentracija padidėjo nuo 63 iki 96 %, palyginus su pradiniu koncentracijų lygiu ir atsižvelgus į eksperimentinių veiksnių derinius.
Tyrimo rezultatai leido padaryti išvadą, kad vasarinių kviečių viso grūdo miltų laikymas kelis mėnesius esant 80 % santykinei oro drėgmei ir 18/28° C temperatūrai neturėjo didelės įtakos DON koncentracijų kitimui. Tačiau vasarinių kviečių miltų laikymo metu DON darinio 3-ADON koncentracijų lygis žymiai sumažėjo, o 15-ADON koncentracijos reikšmingai (beveik du kartus) padidėjo.

Reikšminiai žodžiai: B tipo trichotecenai, drėgmė, efektyvioji skysčių chromatografija, miltai, temperatūra.