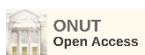




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DEOXYNIVALENOL AND ZEARALENONE CONTENT IN UKRAINIAN COMMON WHEAT (*Triticum aestivum* L.)

Abstract

The study provides a comprehensive assessment of contamination levels of deoxynivalenol (DON) and zearalenone (ZEA) in common wheat (*Triticum aestivum* L.) samples of Ukrainian origin collected over five marketing seasons in 2020–2025. A total of 1250 samples were drawn in accordance with GAFTA No. 124 and ISO 24333:2009 standards. Quantitative analysis was performed using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with isotope-labeled standards. Laboratory testing was carried out by an accredited testing center in accordance with DSTU EN ISO/IEC 17025:2019 and EU Regulation 2023/2782. The results indicate that the overall contamination level generally complies with the requirements of Commission Regulation (EU) 2023/915 and Commission Recommendation 2006/576/EC. The frequency of exceedance of the maximum permissible level for DON (1000 µg/kg) was 1.2%, with the highest rate recorded in the 2021–2022 marketing year (6.43%). For ZEA, exceedances of 100 µg/kg were detected in 0.72% of the samples. It was established that the risk of DON contamination is higher than that of ZEA, although in both cases the vast majority of the samples analyzed remained within permissible limits. Seasonal variability in contamination levels was observed, likely associated with agrometeorological conditions. The obtained results are of practical importance for grain exporters, quality control laboratories, market operators, and grain-processing enterprises, as they may be used to forecast mycotoxigenic risks, improve internal control systems, and ensure consistent compliance with international trade requirements. The findings confirm that Ukrainian common wheat is generally safe with respect to the toxins concerned; however, periodic exceedances highlight the need for continuous monitoring and risk management.

Keywords: mycotoxins, deoxynivalenol, zearalenone, grain, common wheat, safety indicators.

Introduction

Mycotoxins are low-molecular-weight toxic secondary metabolites produced by microscopic fungi, primarily belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Claviceps*. Their formation is influenced by environmental stress factors such as high humidity, temperature, plant damage, and microbial competition. These compounds may accumulate in agricultural commodities during cultivation, harvesting, transportation, processing, and storage.

The presence of mycotoxins in food and feed represents a significant toxicological hazard, causing acute and chronic health effects including carcinogenic, immunosuppressive, nephrotoxic, and hepatotoxic impacts [1]. Among the most relevant mycotoxins in cereals are deoxynivalenol (DON) and zearalenone (ZEA), which are predominantly produced by *Fusarium* species under field conditions.

Due to their thermal stability, these toxins are not effectively eliminated by conventional processing methods [2]. According to the Rapid Alert System for Food and Feed (RASFF), mycotoxins remain one of the leading causes of food safety notifications within the European Union [3].

Given the increasing influence of climate change on fungal contamination patterns, systematic monitoring of mycotoxins in cereal crops is essential to ensure compliance with regulatory standards and to minimize risks to human and animal health.

In the previous article, “Content of Aflatoxins and Ochratoxin A in Ukrainian Wheat” [4], we described in more detail the processes of mycotoxin biosynthesis, factors affecting mycotoxin contamination, etc. We also

considered various characteristics and features of aflatoxins and ochratoxin A and assessed their contamination levels in wheat grain of Ukrainian origin harvested in 2020–2024.

Fusariotoxins are a large group of mycotoxins synthesized by representatives of the genus *Fusarium*, which parasitize mainly cereal crops under field conditions. The most widespread and toxicologically significant fusariotoxins include:

Deoxynivalenol (DON, or vomitoxin) — a type B trichothecene produced by *Fusarium graminearum* and *Fusarium culmorum*. Its main effects in humans and animals include nausea, vomiting, inhibition of protein synthesis, immunosuppression, and damage to the intestinal mucosa [5].

Zearalenone (ZON) — an estrogen-like mycotoxin with hormonal activity. It is produced mainly by *Fusarium graminearum*, *F. culmorum*, *F. equiseti*, and *F. verticillioides*. It is particularly toxic to pigs, causing reproductive disorders, pseudoestrus, and infertility [6].

Fumonisin (mainly type B1) — produced by *Fusarium verticillioides* (formerly *F. moniliforme*) and associated with equine leukoencephalomalacia, pulmonary and hepatic toxicosis in pigs, as well as an increased risk of esophageal cancer in humans [7].

T-2 and HT-2 toxins — type A trichothecenes synthesized mainly by *Fusarium sporotrichioides*, *F. tricinctum*, and *F. poae*. These toxins are highly toxic, exhibiting cytotoxic and immunosuppressive effects and may cause food poisoning, including alimentary toxic aleukia [8].

Most *Fusarium* fungi are typical field pathogens that develop under high humidity and temperate climatic



conditions. A key ecological parameter for the formation of fusariotoxins is the water activity (a_w) of the medium, the minimum value of which is 0.90 for most trichothecene-producing strains [9].

Risk factors include:

- stubble residues and plant debris in the field;
- heavy rainfall during ear formation and ripening;
- minimum tillage;
- wheat cultivar susceptibility to *Fusarium* head blight.

Thus, fusariotoxins are the result of the interaction between the biological properties of phytopathogenic fungi and a complex of agroecological conditions, which determines the need for targeted monitoring at all stages of grain production.

The purpose of the work: to assess the level of mycotoxin contamination in wheat grain of Ukrainian origin harvested in 2020–2024, namely deoxynivalenol (DON) and zearalenone.

Materials and Methods

The object of the study was common wheat grain (1250 samples) grown in different regions of Ukraine in 2020–2024.

Grain samples were collected during the loading of vessel, railcar, truck, and container consignments of Ukrainian common wheat in accordance with GAFTA Sampling Rules No. 124, as well as from grain stores/elevators and vehicles according to ISO 24333:2009 *Cereals and cereal products — Sampling*. Samples provided by representatives of various companies were also analyzed.

The samples were then ground in accordance with the internal working instruction of the Testing Center (TC) of FI “SGS UKRAINE” UA-06365 “Procedure for Preparing Samples for Testing in the FSL Laboratory”. Knife mills FRITSCH Pulverisette 15, Retsch GRINDOMIX GM 200, and GM 300 were used for grinding.

Since the distribution of fungus-infected kernels in a sample can be extremely uneven, the entire available volume of the selected material was used to obtain a high-quality homogeneous sample. The grinding process was combined with homogenization; for this purpose, a riffle divider and GASTROMIX B10C and GOOD FOOD PM 10 planetary mixers were used at different stages of analytical sample preparation.

The content of deoxynivalenol (DON) and zearalenone was determined using high-performance liquid chromatography methods SGS-UA1-0015 and SGS-UA1-0008, respectively. These methods were developed by the specialists of the FSL laboratory of the TC of FI “SGS UKRAINE” and are based on the recommendations of the Phenomenex application note *Mycotoxins Analysis using QuEChERS, SPE and LC/MS/MS*.

The methods are based on the extraction of mycotoxins from the ground sample, purification of the resulting solution using solid-phase extraction technology, and testing of the analytical sample on a liquid chromatograph equipped with a triple quadrupole mass detector. Considering the complexity of the sample matrices, isotope-labeled standards are used to improve the reliability of the results. These standards make it possible to eliminate or reduce background effects and account for possi-

ble deviations during extraction. The measuring equipment used was a Waters Acquity H-Class Plus Xevo TQ-XS LC-MS/MS system.

The described mycotoxin determination procedure complies with the criteria set out in Commission Regulation (EU) 2023/2782 (*Methods of sampling and analysis for the control of the levels of mycotoxins in food*).

To ensure the reliability of testing, the laboratory regularly participates in proficiency testing programs organized by the international provider BIPEA.

The Testing Center of FI “SGS UKRAINE” is accredited in accordance with DSTU EN ISO/IEC 17025:2019. All internal working instructions and methods mentioned above are part of the Quality Management System of FI “SGS Ukraine” and are controlled and updated by the Quality Department of FI “SGS Ukraine”.

Research Results

Control of mycotoxin levels in food and feed is regulated by a number of European Union legal acts mandatory for imports from third countries, including Ukraine. Establishing maximum permissible levels of mycotoxins is a key instrument for reducing the risk of toxicological harm to consumers and animals and for ensuring safe trade in grain products.

Commission Regulation (EU) 2023/915 (consolidated version as of 01.07.2025) defines permissible levels of mycotoxins in unprocessed common wheat intended for human consumption (Table 1). These limits were developed taking into account the toxicological effects of each mycotoxin and consumption standards, especially for vulnerable population groups (children, pregnant women, people with chronic diseases).

The European Commission has also established recommended maximum levels of mycotoxins in common wheat intended for feed purposes in Recommendation 2006/576/EC (consolidated version as of 02.08.2016).

Table 1 – Maximum levels of mycotoxins in common wheat

Mycotoxin	Maximum permissible content (µg/kg), calculated on 12% moisture basis	
	Food purposes	Feed purposes
Deoxynivalenol (DON)	1000	8000
Zearalenone	100	2000

During the 2020–2025 marketing seasons, the content of deoxynivalenol (DON) in common wheat samples demonstrated significant variability (Table 2). Minimum values in all seasons were below 20 µg/kg, while maximum values ranged from 1060 to 5576 µg/kg, exceeding the maximum permissible level of 1000 µg/kg for unprocessed common wheat intended for human consumption. These exceedances mainly occurred in the 2021–2022 marketing season (11 exceedances, 6.43% of the total number of tested samples), whereas in the other seasons there were 1–2 exceedances per season, and in the 2024–2025 marketing season no exceedances were recorded. No exceedances of the recommended level of 8000 µg/kg for feed wheat were detected.



In total, 1,250 common wheat samples were analyzed for DON over five seasons. These results indicate that common wheat contamination with deoxynivalenol (DON) generally remains at a safe level; however, isolated cases of high concentrations pose a serious risk for both food use and export.

Table 2 – Deoxynivalenol (DON) content in common wheat during the 2020–2025 marketing seasons

Mycotoxin / Number of analyzed samples	Min	Max	Average	Median
2020-2021 marketing season				
DON, µg/kg / 177 samples	< 20	1060	171.8	82
Number of samples with exceedances / percentage of total: 1* – 1 / 0.56%; 2* – 0 / 0%				
2021–2022 marketing season				
DON, µg/kg / 171 samples	< 20	5576	316.5	158
Number of samples with exceedances / percentage of total: 1* – 11 / 6.43%; 2* – 0 / 0%				
2022-2023 marketing season				
DON, µg/kg / 456 samples	< 20	3639	111.3	59
Number of samples with exceedances / percentage of total: 1* – 2 / 0.44%; 2* – 0 / 0%				
2023-2024 marketing season				
DON, µg/kg / 421 samples	< 20	2728	54.4	23
Number of samples with exceedances / percentage of total: 1* – 1 / 0.24%; 2* – 0 / 0%				
2024-2025 marketing season				
DON, µg/kg / 290 samples	< 20	639	22	< 20
Number of samples with exceedances / percentage of total: 1* – 0 / 0%; 2* – 0 / 0%				
Summary for 2020–2025 marketing seasons				
DON, µg/kg / 1250 samples	< 20	5576	108,6	33
Number of samples with exceedances / percentage of total: 1* – 15 / 1.2%; 2* – 0 / 0%				

Notes:

1*) Exceedance of the maximum level of deoxynivalenol (DON) (1,000 µg/kg) in unprocessed common wheat for food use according to Commission Regulation (EU) 2023/915.

2*) Exceedance of the recommended level of deoxynivalenol (DON) (8,000 µg/kg) in unprocessed common wheat for feed use according to Commission Recommendation 2006/576/EC.

3) Limit of quantification (LOQ) – 20 µg/kg.

Table 3 – Zearalenone content in common wheat during the 2020–2025 marketing seasons

Mycotoxin / Number of analyzed samples	Min	Max	Average	Median
2020-2021 marketing season				
Zearalenone, µg/kg / 91 samples	< 10	< 10	< 10	< 10
Number of samples with exceedances / percentage of total: 1* – 0 / 0%; 2* – 0 / 0%				
2021–2022 marketing season				
Zearalenone, µg/kg / 90 samples	< 10	168	11.8	< 10
Number of samples with exceedances / percentage of total: 1* – 1 / 1.1%; 2* – 0 / 0%				
2022-2023 marketing season				
Zearalenone, µg/kg / 322 samples	< 10	148	10.5	< 10
Number of samples with exceedances / percentage of total: 1* – 1 / 0.31%; 2* – 0 / 0%				
2023-2024 marketing season				
Zearalenone, µg/kg / 260 samples	< 10	696	16.7	< 10
Number of samples with exceedances / percentage of total: 1* – 4 / 1.54%; 2* – 0 / 0%				
2024-2025 marketing season				
Zearalenone, µg/kg / 208 samples	< 10	194	< 10	< 10
Number of samples with exceedances / percentage of total: 1* – 1 / 0.48%; 2* – 0 / 0%				
Summary for 2020–2025 marketing seasons				
Zearalenone, µg/kg / 971 samples	< 10	696	10.2	< 10
Number of samples with exceedances / percentage of total: 1* – 7 / 0.72%; 2* – 0 / 0%				

Notes:

1*) Exceedance of the maximum level of zearalenone (100 µg/kg) in unprocessed common wheat for food use according to Commission Regulation (EU) 2023/915.

2*) Exceedance of the recommended level of zearalenone (2,000 µg/kg) in unprocessed common wheat for feed use according to Commission Recommendation 2006/576/EC.

3) Limit of quantification (LOQ) – 10 µg/kg.

During the period from 2020 to 2025, the content of zearalenone in common wheat showed substantially lower variability than deoxynivalenol (DON) (Table 3). Minimum values in all seasons were below 10 µg/kg, while maximum values ranged from 148 to 696 µg/kg, exceeding the maximum permissible level of 100 µg/kg

for unprocessed common wheat intended for food use. These exceedances mainly occurred in the 2023–2024 marketing season (4 exceedances, 1.54% of the total number of tested samples); in other seasons there was either one exceedance per season or no exceedances were recorded. No exceedances of the recommended level of 2,000 µg/kg for feed wheat were detected.

In total, 971 common wheat samples were analyzed for zearalenone over five seasons. These results indicate that common wheat contamination with zearalenone generally remains at a safe level; isolated cases of high concentrations were recorded, but the contamination risk is significantly lower than that for deoxynivalenol (DON).

Conclusions

According to the monitoring results for 2020–2025, contamination of common wheat with deoxynivalenol (DON) and zearalenone (ZEA) was detected at generally low levels. This indicates that the risk of contamination with these mycotoxins in the wheat analyzed was minimal and did not pose a significant threat to grain safety under the conditions of the studied region and period. At the same time, the risk of contamination with deoxynivalenol (DON) proved to be more probable under current conditions compared with the risks of contamination with zearalenone (ZEA), which indicates the need for more thorough control.



The obtained results demonstrate interseasonal variability in mycotoxin levels, which correlates with the agrometeorological conditions of particular growing years, especially periods of increased humidity during flowering and grain filling.

The formation of a representative five-year analytical database on the levels of major *Fusarium* toxins in common wheat of Ukrainian origin is of practical significance and may be used to assess export risks, improve internal grain quality control systems, and forecast mycotoxicological hazards depending on climatic conditions.

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ВМІСТ ДЕЗОКСИНІВАЛЕНОЛУ ТА ЗЕАРАЛЕНОНУ В УКРАЇНСЬКІЙ ПШЕНИЦІ (*Triticum aestivum* L.)

Анотація

Дослідження є комплексною оцінкою рівнів забруднення дезоксиніваленолом (DON) та зearаленоном (ZEA) зразків м'якої пшениці (*Triticum aestivum* L.) українського походження, зібраних впродовж п'яти маркетингових сезонів у 2020–2025 роках. Загалом було відібрано 1250 зразків відповідно до стандартів GAFTA № 124 та ISO 24333:2009. Кількісний аналіз проведено за допомогою високоефективної рідинної хроматографії в поєднанні з тандемною мас-спектрометрією (LC-MS/MS) з ізотопно-міченими стандартами. Лабораторні випробування проводилися акредитованим випробувальним центром відповідно до DSTU EN ISO/IEC 17025:2019 та Регламенту ЄС 2023/2782. Результати свідчать про те, що загальний рівень забруднення загалом відповідає вимогам Регламенту Комісії (ЄС) 2023/915 та Рекомендації 2006/576/ЄС. Частота перевищення гранично допустимого рівня для DON (1000 мкг/кг) становила 1,2%, з найвищим показником у 2021–2022 маркетинговому році (6,43%). Для ZEA перевищення 100 мкг/кг виявлено у 0,72% зразків. Встановлено, що ризик забруднення DON є вищим порівняно із ZEA, хоча в обох випадках переважна більшість досліджених зразків залишалася в межах допустимих значень. Спостерігалася сезонна мінливість рівнів забруднення, ймовірно, пов'язана з агрометеорологічними умовами. Отримані результати мають практичне значення для експортерів зерна, лабораторій контролю якості, операторів ринку та підприємств зернопереробної галузі, оскільки можуть бути використані для прогнозування мікотоксикологічних ризиків, удосконалення систем внутрішнього контролю та забезпечення стабільної відповідності вимогам міжнародної торгівлі. Результати дослідження підтверджують, що українська м'яка пшениця загалом безпечна щодо згаданих токсинів; проте періодичні перевищення підкреслюють необхідність постійного моніторингу та управління ризиками.

Ключові слова: мікотоксини, дезоксиніваленол, зearаленон, зерно, пшениця, показники безпечності.

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