TOXICITY OF ZEARALENONE, METHODS OF EXTRACTION FROM VARIOUS PRODUCTS, DETECTION METHODS, AND ITS MIGRATION

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Abstract. Mycotoxins are secondary metabolites of fungi and are among the most widespread groups of contaminants with low molecular weight in food products. These toxins are widespread and can affect the food chain at various stages of plant production, harvesting, storage, and processing. Zearalenone is one of over 400 identified mycotoxins. It is produced by *Fusarium* fungi and primarily exerts estrogenic effects on various organisms. It can cause significant economic losses in products and pose risks to both animals and humans. This article aims to systematize information on the extraction, detection, and migration of zearalenone.

Keywords: mycotoxins, zearalenone, contamination, toxicity, public health, migration.

Annotatsiya. Mikotoksinlar qo‘ziqorinlarning ikkilamchi metabolitlari bo‘lib, oziq-ovqat mahsulotlarida past molekulyar og‘irlikdagi ifloslantiruvchi moddalarning eng keng tarqalgan guruhlari qatoriga kiradi. Ushbu toksinlar keng tarqalgan bo‘lib, o‘simliklarni etishtirish, yig‘ish, saqlash va qayta ishlashning turli bosqichlarida oziq-ovqat zanjiriga ta'sir qilishi mumkin. Zearalenon 400 dan ortiq aniqlangan mikotoksinlardan biridir. Fusarium zamburug‘lari tomonidan ishlab chiqariladi va birinchi navbatda turli organizmlarga estrogenik ta'sir ko‘rsatadi. Bu mahsulotlarda sezilarli iqtisodiy yo‘qotishlarga olib kelishi va hayvonlar va odamlar uchun xavf tug‘dirishi mumkin. Ushbu maqola zearalenonni qazib olish, aniqlash va migratsiyasi haqidagi ma'lumotlarni tizimlashtirishga qaratilgan.

Kalit so‘zlar: mikotoksinlar, zearalenon, ifloslanish, toksiklik, aholi salomatligi, migratsiya.

Аннотация. Микотоксины – вторичные метаболиты грибов и относятся к наиболее распространённым группам низкомолекулярных загрязнителей пищевых продуктов. Эти токсины широко распространены и могут влиять на пищевую цепь на различных этапах производства, сбора, хранения и переработки растений. Зеараленон – один из более чем 400 идентифицированных микотоксинов. Зеараленон продуцируется грибами рода Fusarium и оказывает преимущественно эстрогенное действие на различные организмы. Он может привести к значительным экономическим

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потерям в производстве и представлять опасность как для животных, так и для человека. Целью данной статьи является систематизация информации об извлечении, обнаружении и миграции зеараленона.

Ключевые слова: микотоксины, зеараленон, загрязнение, токсичность, общественное здравоохранение, миграция.

Introduction

Zearalenone (ZEN) is produced by several *Fusarium* species and is a non-steroidal mycotoxin. Its most well-known effect is its estrogenic action, and synthetic derivatives of this mycotoxin, such as zeranol (ZAL), are used in the meat processing industry to accelerate cattle growth. However, this practice is not accepted in the European Union. ZEA is also classified by the IARC as a Group 3 carcinogen, and its negative health effects have been found to cause hormonal imbalances, leading to the development of various reproductive system diseases, such as cervical, ovarian, and prostate cancer. The chemical formula of zearalenone is $C_{18}H_{22}O_5$ (Figure 1), and it is 6-(10-hydroxy-6-oxo-trans-1-undecenyl-beta-resorcylic acid lactone) [2]. It was first isolated from *Fusarium*-contaminated maize (F-2) [3].

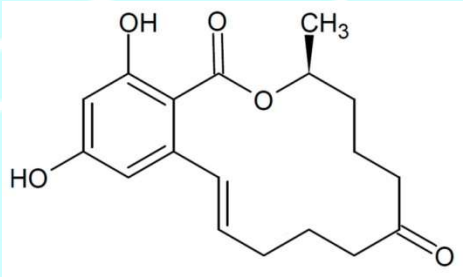


Figure 1. Chemical Structure of Zearalenone

The name "Zearalenone" is derived from the combination of "zea" (from maize, *Zea mays*), "ral" (from resorcylic acid lactone), the suffix "-en" indicating the presence of a ketone group, and the term "one" referring to its ketone group; ZEN is a non-steroidal estrogen mycotoxin biosynthesized through the polyketide pathway [4].

The structure of ZEN resembles naturally occurring estrogens such as estradiol, estrone, estriol, 7b-estradiol, and 17-b-estradiol. It has a molar mass of 318.364 g/mol and is a weakly polar compound in the form of white crystals. ZEN is characterized by blue-green fluorescence when excited at 360 nm and green fluorescence when excited at 260 nm UV light [5]. ZEN has a melting point of 164-165°C. Although it is insoluble in water, it dissolves well in various polar solvents, such as benzene, acetonitrile, acetone, or alcohols. Zearalenone is thermally stable and is not degraded through processing methods such as extrusion, storage, or heating. This mycotoxin primarily accumulates in grains before harvest, but it can also accumulate in poorly stored grains after harvesting [6].

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The conditions for *Fusarium* fungi to produce ZEN are temperatures ranging from 20°C to 25°C and humidity levels above 20%, typically within three weeks. However, fungi also produce ZEN after several weeks when stored under stress or at temperatures between 8°C and 15°C. Research shows that higher levels of zearalenone in grains are commonly found in countries with hot and humid climates [7].

Due to its toxicity, the presence of ZEN in food products has been widely studied. The European Commission has set maximum standards for ZEN levels in selected food products, as shown in **Table 1** [8].

Table-1.

Maximum Zearalenone Standards in Selected Food Products (Commission Regulation (EC) No 1881/2006)

Product	Maximum Permitted Value [µg/kg]
Unprocessed grains other than corn	100
Unprocessed corn	350
Cereals intended for direct human consumption, cereal flour, bran as a final product sold for direct human consumption and germ	75
Refined corn oil	400
Corn and corn-based breakfast cereals intended for direct human consumption	100
Bread (including small bakery products), cakes, biscuits, cereals and breakfast cereals, except corn and corn-based breakfast cereals	50
Processed cereal foods (excluding corn-based processed foods) and baby foods for infants and young children	20
Corn-based processed foods for infants and young children	20

Zearalenone has been frequently detected in various cereal crops such as wheat, barley, maize, sorghum, rye, rice, maize silage, sesame, hay, flour, malt, and soy. Additionally, it can also appear in cereal-based products like those intended for human consumption, baked goods, pasta, and bread. When cows consume food contaminated with ZEA, it can be detected in their milk [9], thus entering the human food chain. Regarding contamination in meat and meat products, a study was conducted on the distribution and detection of ZEA residues in broilers, and it concluded that the levels of this mycotoxin were minimal under experimental conditions. In contrast, research conducted in 2014 [10] highlighted that 52% of the poultry meat samples tested were contaminated with ZEA, with a maximum level of 5.10 µg/kg found in the liver. This emphasizes the need for

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continuous monitoring of these mycotoxins in poultry meat. The toxicokinetics of ZEN mainly involves its absorption, distribution, metabolism, and excretion in the organism. The primary route of entry of ZEN into organisms is through the consumption of contaminated food. In organisms, it can lead to systemic changes through the intestinal microbiota, resulting in the production of various ZEN metabolites [11]. After oral ingestion, ZEN is rapidly absorbed. In the intestines of monogastric animals and the human gastrointestinal tract, ZEN is metabolized by enterocytes into its main metabolites, a- and b-ZEL and a- and b-ZAL, which then undergo biotransformation via two pathways. The first pathway is based on hydroxylation, leading to the formation of a- and b-ZEL by 3a- and 3b-hydroxysteroid dehydrogenases (HSD). The a form has a higher affinity for estrogen receptors, making it more toxic than ZEN itself, while the b form has a lower affinity for these receptors, rendering it nearly harmless. The second biotransformation pathway involves the conjugation of ZEN and its metabolites with glucuronic acid catalyzed by uridine-5-diphosphoglucuronosyltransferase. In humans, ZEN biotransformation occurs primarily in the liver, lungs, kidneys, and intestines [12]. However, in human organisms, ZEN is mostly converted into its a and b isomers through microsomes in the liver, where metabolism is carried out via monohydroxylation through cytochrome P450 (CYP) enzymes [13].

MATERIALS AND METHOD

The research subjects were ZEN solutions purchased as CRM46916 Sigma Aldrich (50 µg/ml) and diluted in acetonitrile to obtain solutions of various concentrations. Chromatographic analysis was performed using the YSSX LC 2030 C 3D Plus device with the RF-20AXS detector, and the wavelength range of 365-465 nm was selected for analysis. A C18 250x4.6 mm 5 µm Precisely (Perkin Elmer) US column was used as the stationary phase. For ZEN analysis, a mobile phase consisting of water (phase A), acetonitrile (phase B), and methanol (phase C) in a constant ratio of 46/46/8 was used. The flow rate was set to 1 ml/min, and the thermostat temperature was 30°C. A 20 µl sample was injected, and the analysis was completed in 15 minutes, yielding the following chromatogram.

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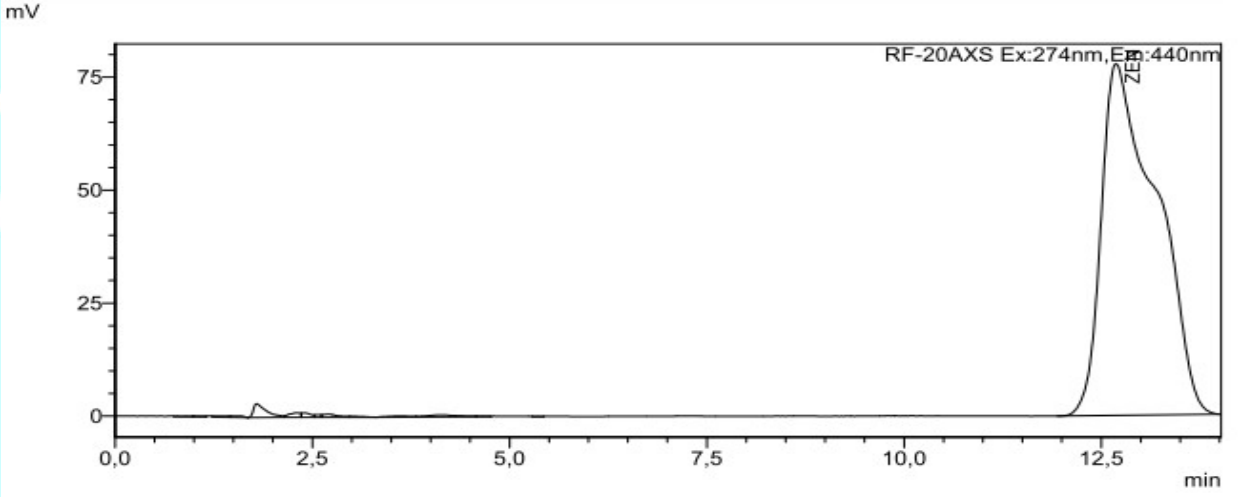


Figure 2. Chromatogram of the zeralenon Standard Sample at a Concentration of 5 µg/ml

A calibration curve was constructed based on standard solutions of zearalenone at concentrations of 0.02, 0.05, 0.1, 0.2, and 5 µg/ml.

Table-2.

Results Obtained from Calibration of Standard Samples.

Standart nomi	**Chiziqli diapazon (µg/ml)**	**Regressiya tenglamasi**	***R*²**
ZON	0,02 -5	f(x)=759677*x-6207,74 Rr1=0,9999984 RSS=3,536810e+007 MeanRF: 6,236524e+005 RFSD: 2,182201e+005 RFRSD: 34,990658	0,9999968

Sample Preparation: For the studies, corn grains contaminated with Fusarium fungus and chickens fed with contaminated feed were obtained. These were prepared for analysis in accordance with the appropriate procedure to detect ZEN mycotoxin. In preparing maize grains for analysis, a 5 g homogenized grain sample was taken and placed in a tightly sealed 50 ml test tube, with 10 ml of water added. The test tube was shaken, and then 10 ml of acetonitrile was added. The mixture was then combined with 2 g of $MgSO_4$ and 1.5 g of NaCl and shaken. The mixture was centrifuged at 4000 rpm for 5 minutes. The resulting solution was treated with 1 g of MgSO4, 0.2 g of silica gel, and 1.00 g of C_{18} sorbent, shaken for 1 minute, and then centrifuged. To detect ZEN, the solvent was evaporated using a rotary evaporator under a nitrogen atmosphere, and then the solution was diluted to 2 ml with the mobile phase [14].

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DISCUSSION OF THE RESULTS

The complexity of food products may hinder the selectivity of Zearalenone detection. Therefore, in addition to applying effective sample pretreatment methods for purification and enrichment before instrumental analysis, choosing an appropriate detection tool is equally important. Currently, LC-FLD is widely used for detecting ZEN in food samples. The optimal chromatographic separation conditions that differentiate the target analyte from the samples are critical to improving analysis selectivity in LC-FLD. When selecting an LC column for detecting ZEN and its metabolites, a C_{18} column is a standard choice. This choice enhances column efficiency and provides satisfactory analytical results, allowing for high precision and narrow chromatographic peaks in a short period of time. The selection of the mobile phase significantly impacts the peak shape and separation effectiveness. Typically, acetonitrile (ACN) and methanol, combined with water, are widely used for reversed-phase chromatographic separation of ZEN and its metabolites. Based on the above conditions, the following chromatograms were obtained for the analysis of Zearalenone in maize and meat.

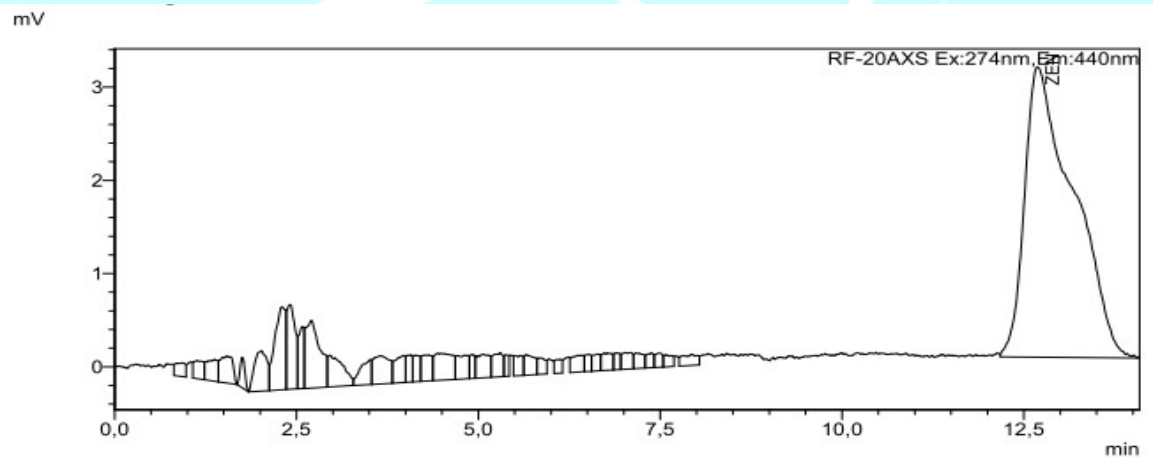


Figure 3. Chromatogram obtained from the analysis of zaralenone in corn.

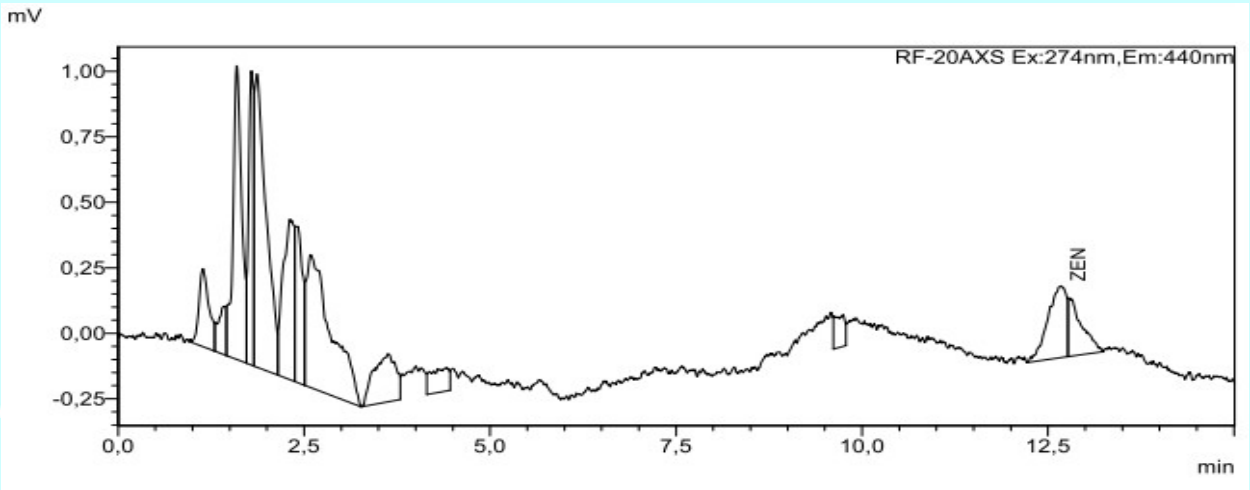


Figure 4. Chromatogram obtained from the analysis of zaralenone in chicken meat.

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Based on the obtained results, the amount of zearalenone detected in maize grains was compared with the amount of zearalenone found in chickens. It was determined that 8.4% of the zearalenone was retained in the chicken liver (Figure 5).

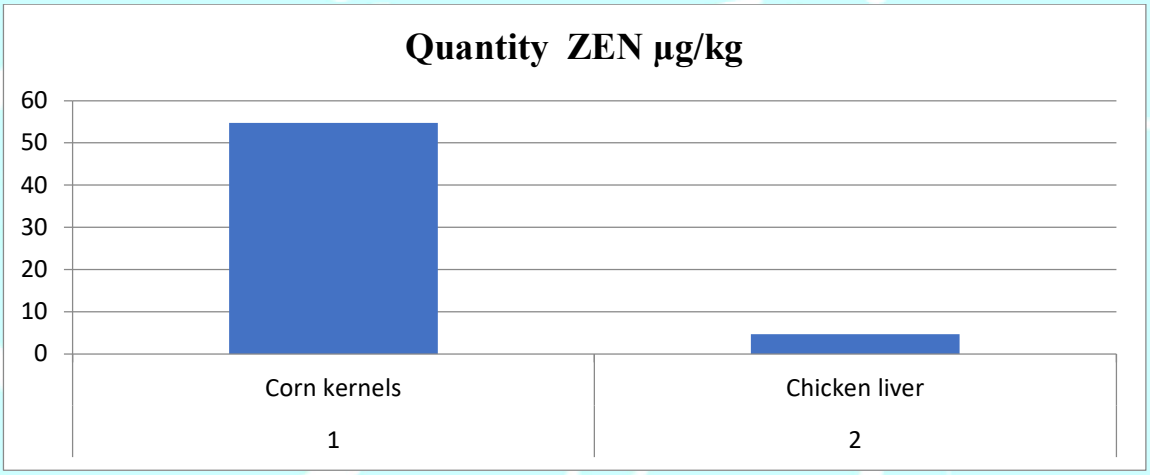


Figure 5. Quantitative analysis results of ZEN in various products using high-performance liquid chromatography (HPLC) Method.

The detection of ZEN and its metabolites in food matrices is an important issue for ensuring food safety standards. However, this task is not straightforward due to specific challenges related to factors such as the precise concentration of ZEN and its metabolites in complex food products, which is further complicated by the presence of interfering compounds. As a result, the need for sample purification and enrichment for quantitative determination becomes a priority, ensuring reliable quantitative and qualitative results.

CONCLUSION Zearalenone is frequently found in feed products and poses a significant risk in animal husbandry. Its negative effects on reproduction are well-studied. Animal breeders worldwide are experiencing economic losses due to their lack of experience in detecting and decontaminating contaminated feed products. Recent literature has highlighted the negative impact of ZEN on immunity and gut health. In this context, the migration process of zearalenone in various products has been studied.

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