1.1.1.2. pasākuma "Pēcdoktorantūras pētniecības atbalsts" ietvaros apstiprināts pētniecības projekts Nr.1.1.1.2/VIAA/1/16/219 „Masspektrometrijas metožu izstrāde un pielietojums regulēto un mazizpētīto mikotoksīnu satura novērtējumam Latvijas lauksaimniecības un pārtikas produktos prioritārās bioekonomikas nozares attīstības atbalstīšanai un attīstīšanai"

Research project No. 1.1.1.2/VIAA/1/16/219 "Development and application of mass spectrometric methods for the occurrence assessment of regulated and emerging mycotoxins in agricultural and dairy products in Latvia, providing contribution to the priority bio-economy sectors" under activity 1.1.1.2. "Post-doctoral Research Aid"

ZINĀTĪBA (KNOW-HOW)

Gamma starojuma pielietojums mikotoksīnu piesārņojuma kontrolei graudu un citu lauksaimniecības produktu kultūrās
Application of gamma radiation for the control of mycotoxin contamination in grains and other agricultural crops

3. aktivitāte "Vides, fizikāli-ķīmiskie un tehnoloģiskie faktori", rezultatīvais rādītājs D 3.1
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Izstrādāts: Pārtikas drošības, dzīvnieku veselības un vides zinātniskais institūts "BIOR"
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I. Reinholds

SATURS

(CONTENTS)

Abbreviations (Saīsinājumi)

15-AcDON – 15-acetildeoksinivalenols (15-acetyldeoxynivanelol)
15-MAS – 15-monoacetoksiskripenols (15-monoacetoxyscripenol)
3-AcDON – 3-acetildeoksinivalenols (3-acetyldeoxynivanelol)
AF – aflatoksīni (aflatoxins)
AFB_1 – aflatoksīns B_1 (aflatoxin B_1)
AFB_2 – aflatoksīns B_2 (aflatoxin B_2)
AFG_1 – aflatoksīns G_1 (aflatoxin G_1)
AFG_2 – aflatoksīns G_2 (aflatoxin G_2)
AFL – aflatoksikols (aflatoxicol)
ALT – altenuēns (altenuene)
AME – alternariola monometilēteris (alternariol monomethyl ether)
AOH – alternariols (alternariol)
ATX I – altertoksīns I (altertoxin I)
ATX I – altertoksīns I (altertoxin I)
D(abs) – absorbētā doza (absorbed dose)
D3G – deoksinivalenola-3-glikozīds (deoxynivalenole-3-glycoside)
DON – deoksinivalenols (deoxynivalenol)
EK (EC) – Eiropas Komisija (European Commission)
ENN A – enniatins A (Enniatin A)
ENN A_1 – Enniatin A_1
ENN B – Enniatin B
ENN B_1 – Enniatin B_1
FB_1 – fumonizīns B_1 (Fumonisin B_1)
FB_2 – fumonizīns B_2 (Fumonisin B_2)
FB_3 – fumonizīns B_1 (Fumonisin B_1)
FUS X – fusarenons X (fusarenon X)
HPLC – augstas izšķirtspējas masspektrometrija (high performance liquid chromatography)
HT-2 – HT-2 toksīns (HT-2 toxin)
JS (IR) – jonizējošais starojums (ionising radiation)
kGy – kilogrejs (kilogray)
MPA – mikofenolskābe (mycophenolic acid)
Mycophenolic acid MPA
NEO – neosolaniols (neosolaniol)
NIV – nivalenols (nivalenol)
Orbitrap-MS – Orbitrtap masspektrometrija (Orbitrap mass spectrometry)
OTA – ohratoksīns A (ochratoxin A)
OTB – ohratoksīns B (ochratoxin B)
STC – sterigmatocistīns (sterigmatocystin)
T-2 – T-2 toksīns (T-2 toxin)
T-2TETR – T-2 tetraols
T-2TRI – T-2 triols
TEN – tentoksīns (tentoxin)
ZEN – zearalenons (zearalenone)

1. Foreward (Priekšvārds)

Mycotoxins, secondary metabolites of molds, are among the most toxic contaminants in food and feed that can accumulate in various crops during their growth, harvesting and storage stages. Depending on climatic conditions, agricultural products may contain different mycotoxins. *Fusarium* fungi and their produced mycotoxins (class A and B trichothecenes: T-2 and HT-2 toxins, and deoxynivalenol (DON)), zearalenone (ZEN), etc.) are the predominant contaminants of cereals grown in Latvia and neighbouring European countries. Research on unregulated *Fusarium* and *Alternaria* mycotoxins and derivatives of regulated mycotoxins (masked compounds, secondary metabolites, etc.) has become crucial in recent years. The grain storage process identifies a potential risk of mycotoxin contamination that leads to research of mitigation methods for mycotoxin control in grains.

Nowadays, several known physico-chemical methods have been used to reduce the contents of mycotoxins in food products - thermal treatment, ozonation, treatment with non-ionizing and ionizing radiation methods (e.g., cold plasma, UV radiation, ionizing radiation) are some of the examples. Ionizing radiation methods providing gamma, X-ray, or acelerated electron radiation are still used within sterilisation of various foodstufs.

In most of the reported studies of ionising radiation source applications only radiation stability of one to few mycotoxins in artificially contaminated samples have been reported.

There is a lack of studies on the effects of ionizing radiation on the stability of several unregulated and regulated mycotoxins in naturally contaminated solid food matrix samples, in particular the post-irradiation properties of multi-class mycotoxins have been also little evaluated.This know-how summarizes the results of studies on the effects of gamma radiation on the mycotoxin content of agricultural products. The description of know-how consists of the following sections, written in English:

- 2. Technological background
- 3. Experimental section
- 3.1. Test samples
- 3.2. Gamma radiation treatment
- 3.3. Instrumental analysis
- 4. Mycotoxin stability after treatment by ionizing radiation
- 4.1. Regulated mycotoxin (DON, ZEN, T-2) standard solutions
- 4.2. Multi-mycotoxin stability in irradiated grain samples
- 5. Conclusions

This know how description has one MS Excel attachment – a more detailed data set of the results presented in this report.

Mikotoksīni, pelējuma sēņu veidotie sekundārie metabolīti, ir vieni no toksiskākajiem pārtikas un dzīvnieku barības piesārņotājiem, kas var uzkrāties dažādās lauksaimniecības kultūrās to augšanas, novākšanas un uzglabāšanas posmos. Atkarībā no klimatiskajiem apstākļiem, lauksaimniecības produkti var saturēt atšķirīgus mikotoksīnus. Latvijā un Eiropas kaimiņvalstīs galvenokārt raksturīga *Fusarium* pelējuma sēņu un attiecīgi to veidoto mikotoksīnu (A un B trihotecēnu: T-2 un HT-2 toksīnu, deoksinivalenola (DON), zearalenona (ZEN) u.c.) izplatība graudaugos. Pēdējos gados aktuāla kļuvusi neregulētu *Fusarium* un *Alternaria* mikotoksīnu un regulēto mikotoksīnu atvasināto formu (maskētas formas, sekundārie metabolīti u.c.) izpēte. Graudu uzglabāšanas process nosaka potenciālu risku mikotoksīnu tālākai attīstībai, tādēļ tiek meklēti paņēmieni mikotoksīnu kontrolei graudos.

Mūsdienās ir zināmas vairākas fizikāli-ķīmiskās metodes, kas tiek lietotas mikotoksīnu satura mazināšanai pārtikas produktos - termiskā apstrāde, ozonēšana, apstrāde ar nejonizējošā un jonizējošā starojuma metodēm (aukstā plazma, UV starojums, jonizējošais starojums). Jonizējošā starojuma metodes kā gamma, rentgena, vai paātrinātu elektronu starojums joprojām tiek pielietotas dažādu pārtikas produktu sterilizācijai.

Galvenokārt zināmie pētījumi ietvēra viena vai dažu mikotoksīnu stabilitātes izpēti, veicot mākslīgi piesārņotu paraugu apstarošanu ar jonizējošā starojuma avotu.

Trūkst pētījumu par jonizējošā starojuma iedarbību uz vairāku neregulēto un regulēto mikotoksīnu stabilitāti dabiski kontaminētos cieto pārtikas matricu paraugos, it īpaši multi-klases mikotoksīnu īpašībām pēc starošanas.

Šajā zinātības aprakstā ir apkopoti pētījuma gaitā iegūtie rezultāti par gamma starojuma ietekmi uz mikotoksīnu saturu lauksaimniecības produktos. Zinātības aprakstu veido sekojošas nodaļas, kas rakstītas angļu valodā:

- 2. Technological background
- 3. Experimental section
- 3.1. Test samples
- 3.2. Gamma radiation treatment
- 3.3. Instrumental analysis
- 4. Mycotoxin stability after treatment by ionizing radiation
- 4.1. Regulated mycotoxin (DON, ZEN, T-2) standard solutions
- 4.2. Multi-mycotoxin stability in irradiated grain samples
- 5. Conclusions
- References

Zinātības aprakstam pievienots MS Excel datu kopa, kas satur detalizētu pārskatu, par pētījuma rezultātiem.

2. Technological background

Several technological solutions are available for ionizing radiation sterilization of food samples, that include X-rays, accelerated electrons (generated by linear electron accelerators - LINAC) and gamma radiation sources (gamma quanta are generated by cobalt-60 (Co-60), or less frequently by Cs-137 isotope generators). All mentioned sources have similar mechanism of irradiation of matter, whereas radiolysis process involves generation of primary or secondary (X-ray, gamma quanta) electrons and further radiolysis reactions. The main difference between the different irradiation technologies is attributed to the radiation energy, and radiation absorption rate, affected by several factors: sterilization time, particle penetration depth, irradiation temperature and atmosphere. Limitations are observed in the case of accelerated electron radiation, because the low electron scattering and sorption do not effectively sterilize large specimens - there is not sufficient penetration depth and uniform absorption throughout the volume to be sterilized. The Most frequently used method for food sterilisation is the technology based on Co-60 isotope source generated gamma radiation generating photon radiation of 1.17-1.33 MeV energy, that ensures deep penetration efficiency [1].

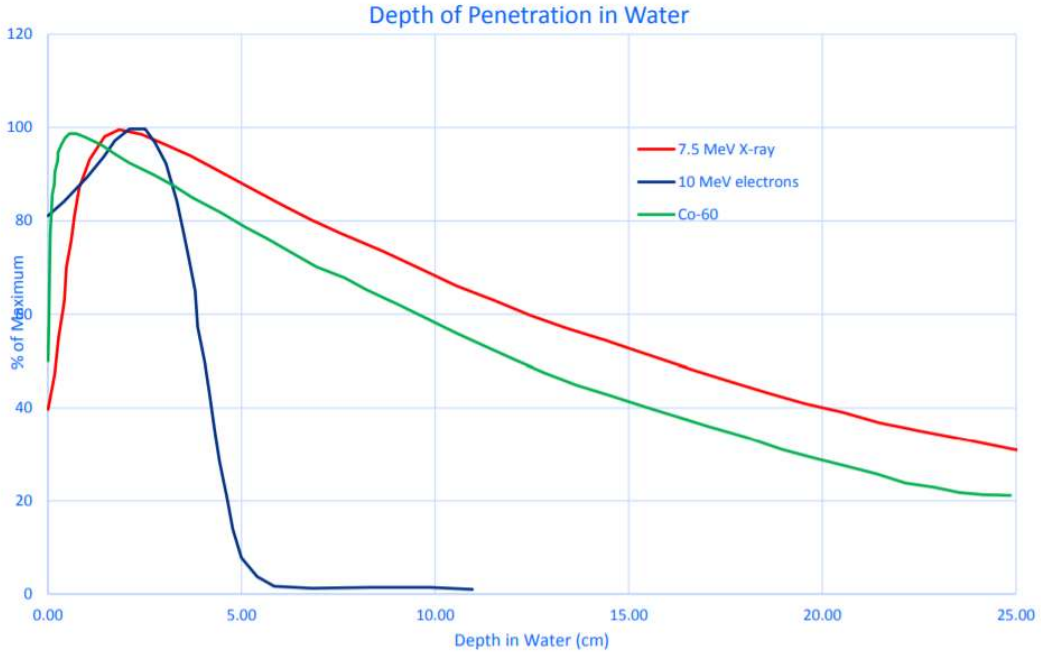

Fig. 1. Comparison of penetration depth of X-ray, g photon and accelerated electron radiation [1].

In this study mitigation of mycotoxin contamination in different naturally contaminated agricultural cultivars was evaluated using gamma radiation treatment. The conclusions of radiation efficiency were provided.

3. Experimental section

3.1. Test samples

Six agricultural cultivars included four wheat samples, one barley and one broad bean sample were obtained from Latvian harvesters and used in this study (Table 1). The samples each with a mass of approximately 1 kg were ground in a knife mill, placed in sterile laboratory sample bags and kept in dark at -20°C until further activities. The samples were not artificially contaminated with mycotoxin standards. The natural contamination levels determined by multi-method were evaluated in the samples control samples before their irradiation by gamma rays.

Table 1. Sample matrices sterilised by gamma rays.

Sample	Acronym	Moisture (%)	Water activity (aw)
Wheat 1	WA	14.2	0.57
Wheat 2	WB	12.8	0.43
Wheat 3	WC	14.0	0.59
Feed wheat	FW	14.1	0.58
Barley	BA	14.6	0.47
Broad beans	BB	11.1	0.49

3.2. Gamma radiation treatment

Preparation of samples for the irradiation. The ground samples were placed in sterile polyethylene bags. It was noted that packages need to be sealed enough to protect the sample from outer contamination, but at the same time not too tight taking into account gaseous products that may be formed during air radiolysis, which in the case of an overly sealed sample may affect the opening of the package.

Irradiation conditions. The samples were irradiated at room temperature in air with gamma rays. Irradiation was performed using the TBI 8450 – 150 tote boxes and irradiated in the batch type gamma irradiation facility at Scandinavian Clinics Estonia (Steri, Estonia). Irradiations were done according to ISO 11137:2015 and ISO 13485:2016 requirements.

Radiation parameters are described in Table X. Dosimetry monitoring is crucial to assess the actual dose absorbed by the radiation. Various commercially available radio chromic polymer film dosimeters are suitable for this purpose, which can be easily attached to the radiating materials and provide an accurate determination of the true value of the absorbed dose according to the absorbed radiation and the data of the calibration graph. In this case, a dosimeter system (WinDose radio chromosome dosimeter, GEX Corporation, USA) was used to control the radiation dose.

Figure 2. Irradiation conditions: tote box-based facility: boxes routed to zone of irradiation (a), dosimeters attached to irradiated boxes for absorbed dose monitoring after the sterilisation (b)

Table 2. Irradiation parameters.

Dose (kGy)	**Average dose rate (kGy/h)**	**(Range of absorbed doze (KGy)**
5	3.25	4.8-5.2
7	2.00	6.8-7.2
10	2.10	9.50-10.5
15	2.30	14.3-15.3
25	2.28	25.0-26.2

3.3. Instrumental analysis

Reagents. All the reagents need to be of high analytical quality (ACS or similar). Mycotoxin standards (purities > 97%) where obtained from Biopure (MA, USA), Cayman Chemical (MI, USA and (Fermentek Ltd. (Israel). Stock solutions at 1000 mg/L were prepared in non-aqueous solvents and stored in freezer at - 20°C. Working standard solutions were prepared in 20% aqueous acetonitrile and

stored in amber glass bottles, in freezer at -20°C. All the solvents were of ACS grade and proper for the instrumental analysis of mycotoxins.

Table 3. Mycotoxins analysed in grain samples (* - regulated in grains of food and feed [2-4]).

Mycotoxin	***Mycotoxin type/ Fungi genera***
AFB1	*Aflatoxins / A. spp. (Aspergillus flavus, Aspergillus parasiticus)*
AFB2	*Aflatoxins / A. spp. (Aspergillus flavus, Aspergillus parasiticus)*
AFG1	*Aflatoxins / A. spp. (Aspergillus flavus, Aspergillus parasiticus)*
AFG2	*Aflatoxins / A. spp. (Aspergillus flavus, Aspergillus parasiticus)*
AFL	*Aflatoxins / A. spp. (Aspergillus flavus, Aspergillus parasiticus)*
OTA*	*Ochratoxins / Penicillium spp., A. spp.*
OTB	*Ochratoxins / Penicillium spp., A. Sspp.*
15-MAS	*Type A Trichothecene/ Fusarium spp.*
T-2*	*Type A Trichothecene/ Fusarium spp.*
HT-2*	*Type A Trichothecene/ Fusarium spp.*
T-2TRI	*Type A Trichothecene/ Fusarium spp.*
T-2TETR	*Type A Trichothecene/ Fusarium spp.*
NEO	*Type A Trichothecene/ Fusarium spp.*
NIV	*Type A Trichothecene/ Fusarium spp.*
DON*	*Type B Trichothecenes/ Fusarium spp.*
D3G	*Type B Trichothecenes/ Fusarium spp.*
3-AcDON	*Type B Trichothecenes/ Fusarium spp.*
15-AcDON	*Type B Trichothecenes/ Fusarium spp.*
FUS X	*Type B Trichothecenes/ Fusarium spp.*
ZEN	*Fusarium mycotoxin / Fusarium spp.*
ENN B	*Emerging Fusarium mycotoxin/ Fusarium spp.*
ENN B1	*Emerging Fusarium mycotoxin/ Fusarium spp.*
FB1	*Fumonisin/ Fusarium spp.*
FB2	*Fumonisin/ Fusarium spp.*
FB3	*Fumonisin/ Fusarium spp.*
MPA	*Penicillium sp. / Penicillium brevicompactum*
TEN	Alternaria toxins /Alternaria alternaria
ATX I	Alternaria toxins /Alternaria alternaria
AOH	Alternaria toxins /Alternaria alternaria
AME	Alternaria toxins /Alternaria alternaria
ALT	Alternaria toxins /Alternaria alternaria

Standard mixture solutions of three main regulated mycotoxins (DON, ZEN, T-2) at their relative contamination proportions were prepared in acetonitrile to evaluate radiation stability of pristine mycotoxins. Three standard mixture solutions namely control, 10 kGy and 25 kGy solutions were prepared at 1.5 mL volumes and each contained following concentrations of these mycotoxin standards per each of the solutions: DON (100 ng/g), ZEN (20 ng/g) and T-2 toxin (10 ng/g), respectively.

Mycotoxin solutions in water were not analysed. It is well known that reactions of radiolysis may be relatively fast in water leading to high degradation rate of mycotoxins, that has been reported

in many studies. The aim of this report was to evaluate the emerging mycotoxins in real samples and stability of these low concentration toxins after treatment by gamma rays. For this purpose, it was crucial to use HPLC equipped with high resolution MS detector, thus, a validated method based on HPLC equipped with Orbitrap-MS detection was developed and applied.

Sample preparation was provided based on the QuEChERS extraction method with freeze out step after extraction with the following steps:

- Adding a mixture of 10 mL of water with 2% formic acid and 10 mL of acetonitrile to sample (5 g) and shaking;
- Adding a QuEChERS salt mixture of 4.0 g of anhydrous MgSO4, 1.0 g of NaCl, 1.0 g of trisodium citrate and 0.5 g of disodium citrate followed by shaking and centrifugation;
- Separation of the organic layer and storage of it in a freezer at -80°C for 30 min;
- Centrifugation;
- Preconcentration of the extracts after freezing out by a factor of 5 using evaporation under a stream of nitrogen at 50°C;
- Reconstituting in 10% aqueous acetonitrile containing 0.1% formic acid and filtration and transfer of the extracts to chromatographic vials for the analysis.

The main parameters of instrumental conditions of the method are included in Table 4.

Table 4. Main instrumental performance parameters of mycotoxin analysis.

HPLC conditions	
Column	C18 (150 × 2 mm; 3 μm)
Ionization mode	Electrospray (HESI-II)
Mode	Positive (+3.5 kV) / Negative (-2.5 kV)
Injection volume	40 μL
Mobile phases	A: H2O+0.1% FA; B: Acetonitrile + 0.1% FA
Orbitrap MS conditions	
MS mode	Parallel reaction monitoring
Resolution	70 000
Injection time	1000 ms
Isolation window	0.4 m/z

Quality assurance / quality control. The quality control criteria for positive identification of the analysed mycotoxins include the retention time of the native compounds. Analytes were confirmed by the presence of overlapping peaks from at least two different product ions possible. Sum of all observed fragments was used to construct extracted ion chromatograms for the integration. Six-point calibration curves must be used for the quantification of analyte concentrations in each sample run.

Procedural blanks must be included in the quality control (QC) protocol and must be analysed in each sample sequence during the validation procedures and analysis of certified reference materials. The

concentrations of contaminants determined in the analysed samples are corrected by taking into account the analyte concentrations found in procedural blanks. To evaluate the method reliability for the detection and quantification of mycotoxins, the selectivity, linearity, recovery, and repeatability are verified. Evaluation of LOQ was based on S/N≥10. In some cases, mycotoxin concentrations at the detection level (LOD = LOQ/3) were determined. For estimation of measurement uncertainty, a total of 3 procedural replicates were analyzed over three different days for the following representative matrices – wheat, barley, beans. The average expanded measurement uncertainty in the case of most determined mycotoxins was below 20%.

4. Mycotoxin stability after treatment by ionizing radiation

The mycotoxin compounds included in the method are summarised in Table 3. To best of our knowledge, AFB_1, OTA, ZEN, and DON in grain, spices, coffee, etc. products as well as patulin formed in apple juices are the major mycotoxins included in the studies of gamma radiation treatment for the control of fungi and their produced metabolites [5-9]. However, most of the studies were based on the artificially contaminated products. The efficiency of gamma radiation on emerging *Fusarium* and *Alternaria* mycotoxin degradation has not been studied while this topic may be of high interest.

Despite of increasing rate of research studies and industrial applications of gamma radiation sources used in food treatment in many non-European countries and European countries rich in nuclear facilities, the application of these sources are rather limited to only few food products and have stringent regulations for application only for sterilisation of dried aromatic herbs, spices and vegetable seasonings with the maximum allowed absorbed dose of irradiation up to 10 kGy [10].

There are several recent reports and overviews ensuring the topical notability of gamma sterilisation and other radiation treatment methods for safe non-thermal irradiation of foodstuffs using doses even up to the traditionally used dose of the irradiation (25 kGy) [11-15].

In the current study doses from 5 kGy (determined as successful level of ionising radiation energy for the mitigation of mould contents [16]) up to the traditionally used sterilisation dose of food packaging materials (25 kGy) were evaluated.

4.1. Regulated mycotoxin (DON, ZEN, T-2) standard solutions

The results of mycotoxin standard levels in acetonitrile prior and after irradiation to 10 and 25 kGy are expressed in Table 5 and indicate rather low rate of trichothecene degradation (<50%) as compared to cyclic ZEN structure. One of the reasons for higher radiation stability of trichothecenes may be related to aromatic backbone reducing efficiency of radiation susceptibility through the energy dissipation by resonance within the benzene ring structure.

Table 5. Main instrumental performance parameters of mycotoxin analysis.

	Determined mycotoxin concentrations (ng/g)		
Dose (kGy)	DON	ZEN	T-2
0 (Control)	97	21	8.5
10	87	3.7	8
25	54	0.13	6.9
Degradation efficiency at 10 kGy (%)	10.31	82.38	5.88
Degradation efficiency at 25 kGy (%)	44.33	99.38	18.82

The chromatographic peaks reported in Fig. 3-4 clearly indicate the presence of mycotoxins in the control samples and the reduction of peak intensities within the rise of irradiation dose.

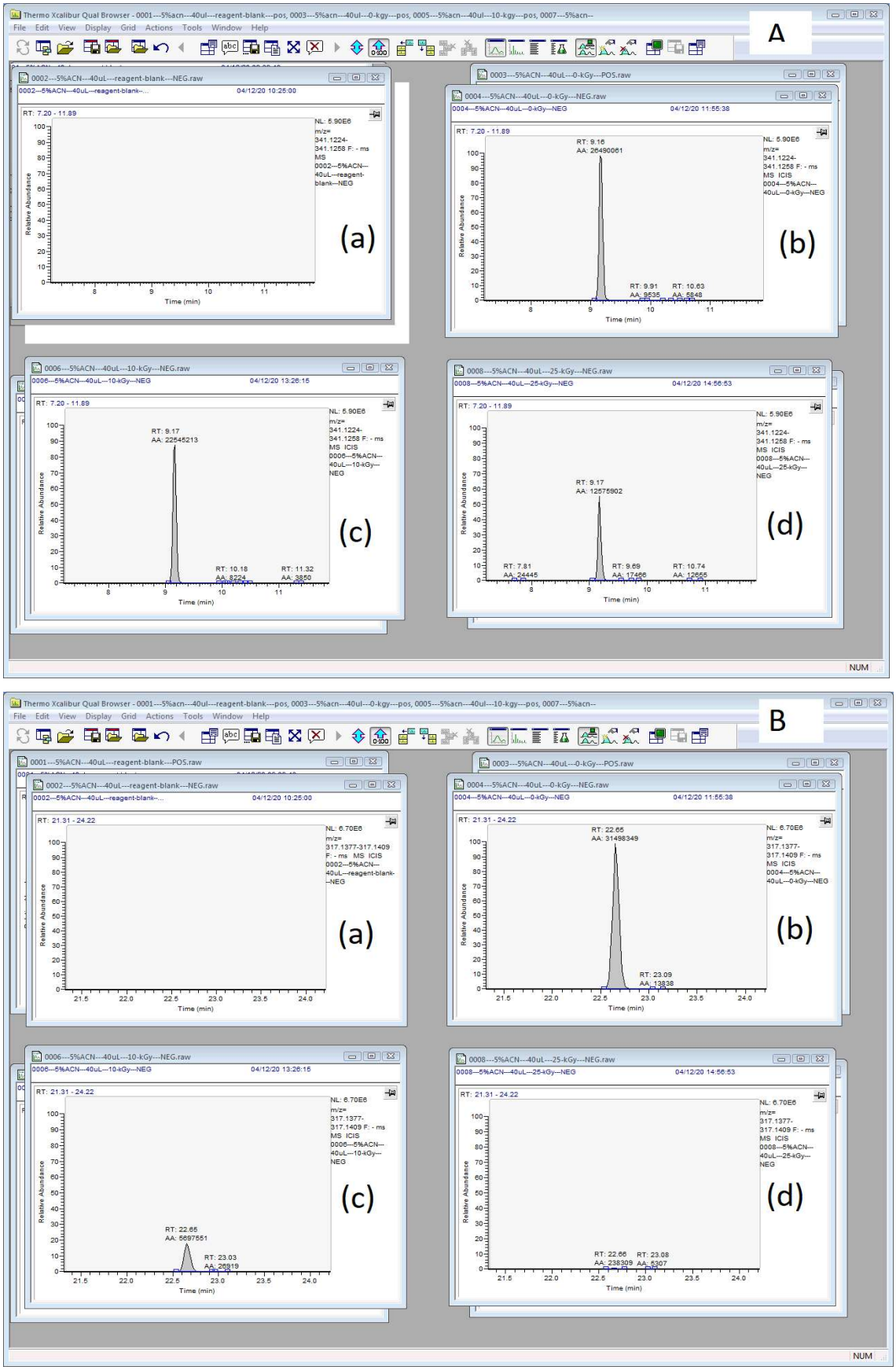

Figure 3. Chromatograms confirming the changes of irradiated mycotoxin (A: DON, B: ZEN) standard solutions in ACN after irradiation to 10 and 25 kGy (a - blank, b - 0 kGy (control), c - 10 kGy, d - 25 kGy).

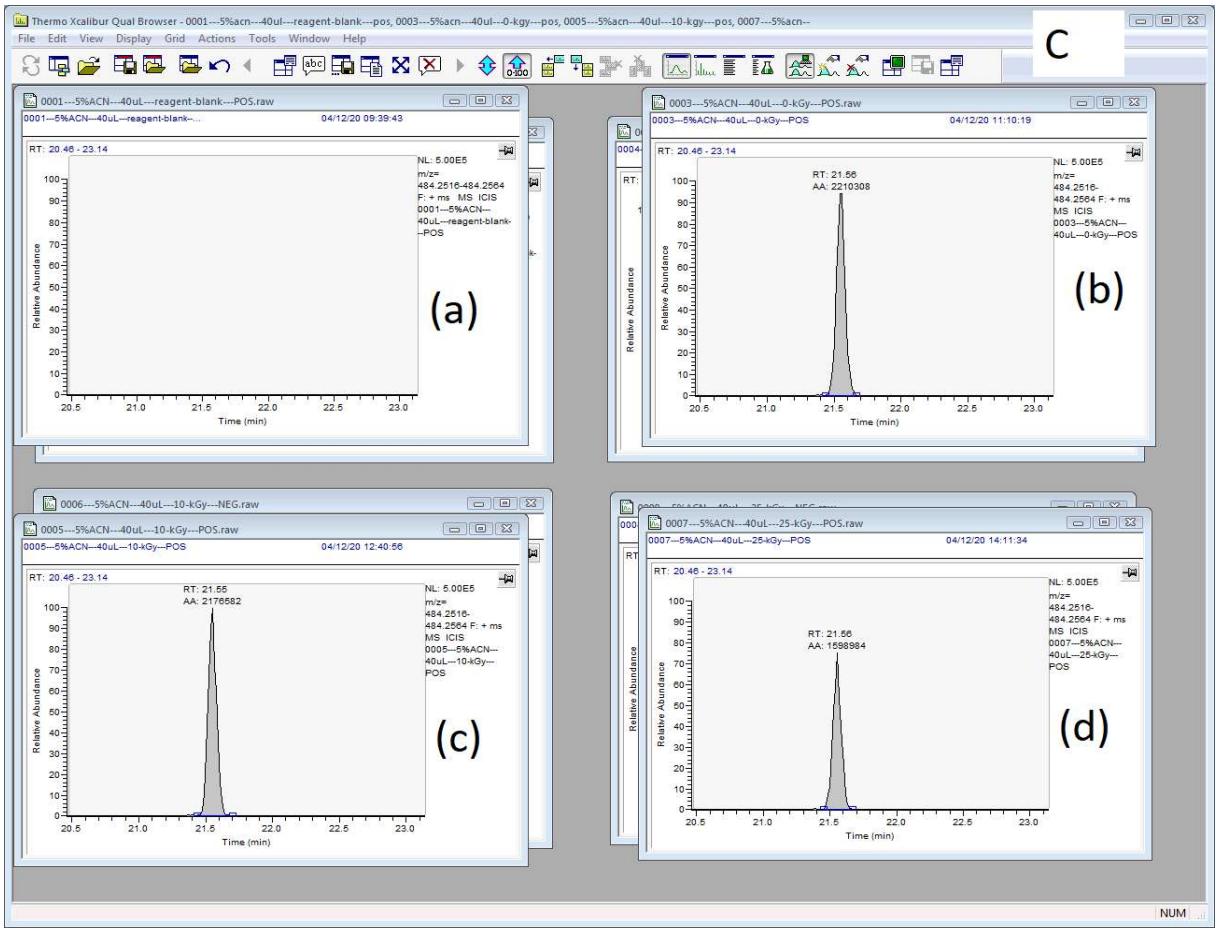

Figure 4. Chromatograms confirming the changes of irradiated T-2 standard solutions in ACN after irradiation to 10 and 25 kGy (a - blank, b - 0 kGy (control), c - 10 kGy, d - 25 kGy).

The non-aquatic matter allows to evaluate maximally the radiation stability of mycotoxins. However, in the case of food matrices the effect of water contents may affect the rate of radiolytic processes.

4.2. Multi-mycotoxin stability in irradiated grain samples

While most of the studies commonly include evaluation of stability in the case of artificially contaminated samples, the purpose of the know-how was to evaluate natural contamination and degradation of determined mycotoxins. Application of high-resolution mass spectrometry methods such as Orbitrap-MS allows to determine mycotoxins at low concentrations. However, the detection and quantification levels should be taken into account, when evaluating the real degradation efficiency after radiation treatment.

All the results are included in the attached data base, whereas only the main findings will be included in the report paper.

Control samples. Instrumental method allowed to determine 6 to 16 mycotoxin compounds per sample. First it was checked whether the carcinogens (AFs, OTA, FB1 + FB2) could be found in the samples.

Aflatoxins were not determined in any of the tested control samples, whereas 12 ng/g of aflatoxin metabolite (AFL) was found in the bean sample, which was degraded at least by 60% after irradiation to doses > 10 kGy (contents of AFL reduced below 5 ng/g).

OTA (14 ng/g) and OTB metabolite (0.6 ng/g) were determined in the feed wheat sample. The EC Directive has set 250 ng/g as the maximum content of OTA in cereals and cereal products used as feed [4], thus, this level was not increased.

It was determined that also natural levels of regulated *Fusarium* mycotoxins determined in three control wheat, one feed wheat, one barley and one broad bean samples were far below the maximum concentrations limited as safe for human beings and animals according to the set regulations in Europe [2-4].

Broad beans were less contaminated as the sample contained only six mycotoxins: AFL, NIV, MPA, TEN, AOH, AME, ALT. Feed wheat samples contained 7 compounds: OTA, OTB, T-2, ENN B, MPA and TEN *Alternaria* toxin.

Edible wheat samples contained 10 to 13 mycotoxins, but barley samples were most contaminated with up to 16 mycotoxins. Class A and B trichothecenes, ENNs and *Alternaria* toxins predominated in both wheat and barley samples.

Irradiated samples: stability of regulated mycotoxins and their derivatives. First of all, the degradation rate of the most regulated mycotoxins (DON, T2, HT-2 and ZEN, OTA) where evaluated in the irradiated naturally contaminated samples.

As noted, only the FW sample contained OTA and its dechlorinated metabolite, which were degraded notable even at 10 kGy dose: the degradation rate of OTA reached 57% at this dose and increased up to 60% at 25 kGy, respectively. Similar effect was determined in the case of OTA.

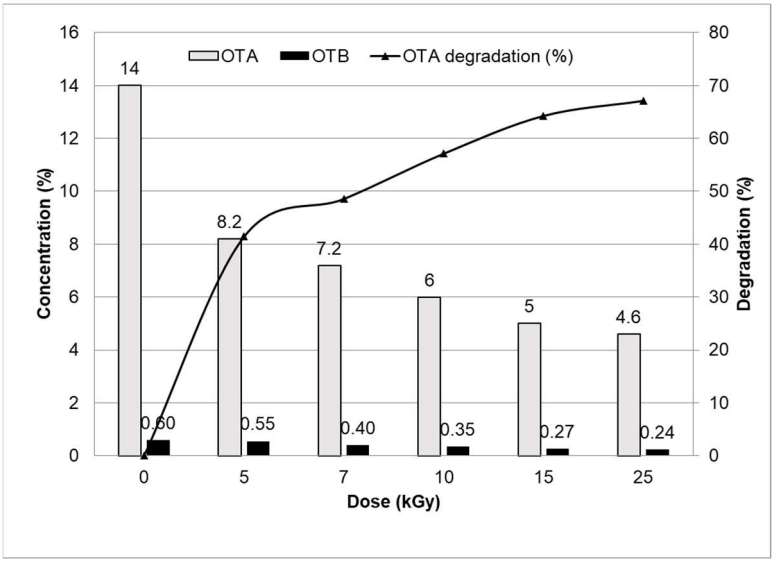

Figure 5. OTA and OTB degradation in FW as dependence of the absorbed irradiation dose.

Tests of standards indicated to rather high degradation rate of ZEN even at 10 kGy dose. The real grain samples mostly were free of ZEN with exception of one edible wheat (WB), which presented co-occurrence of OTA, ZEN and other contaminants. The degradation rate of ZEN in FW was similar to that determined in case of ZEN standard solutions in ACN: mycotoxin level reduced by more than 70% at 10 kGy dose. The study indicated that increase of dose had little effect on further degradation reactions (Figure 6.).

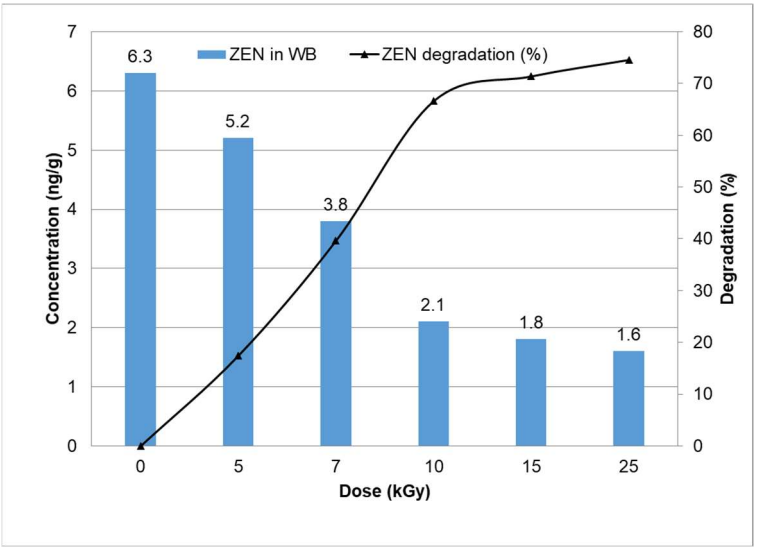

Figure 6. ZEN degradation in FW as dependence of the absorbed irradiation dose.

T-2 and its hydrolysed metabolite (HT-2) are the major toxic compounds regulated in cereals. The characteristic degradation curves of T-2 and HT-2 toxins in cereals are shown in Figure 7.
These data are in good agreement with the results determined in the case of T-2 standards and indicate rather low susceptibility to radiation treatment at doses < <10 kGy.

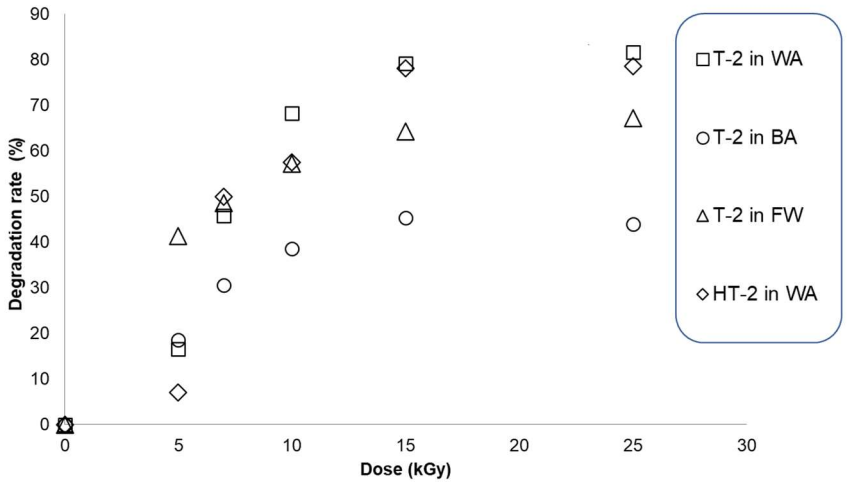

Figure 7. Characteristic T-2 toxin and HT-2 degradation rate in different grain matrices as dependence of the absorbed irradiation dose.

Figure 8. indicate the decomposition rate of T-2 and HT-2 toxins, which showed higher contents to that of T-2 in most cases of the analysed samples with exception of FW. It was also determined that BB showed no prevalence of A class trichothecenes.

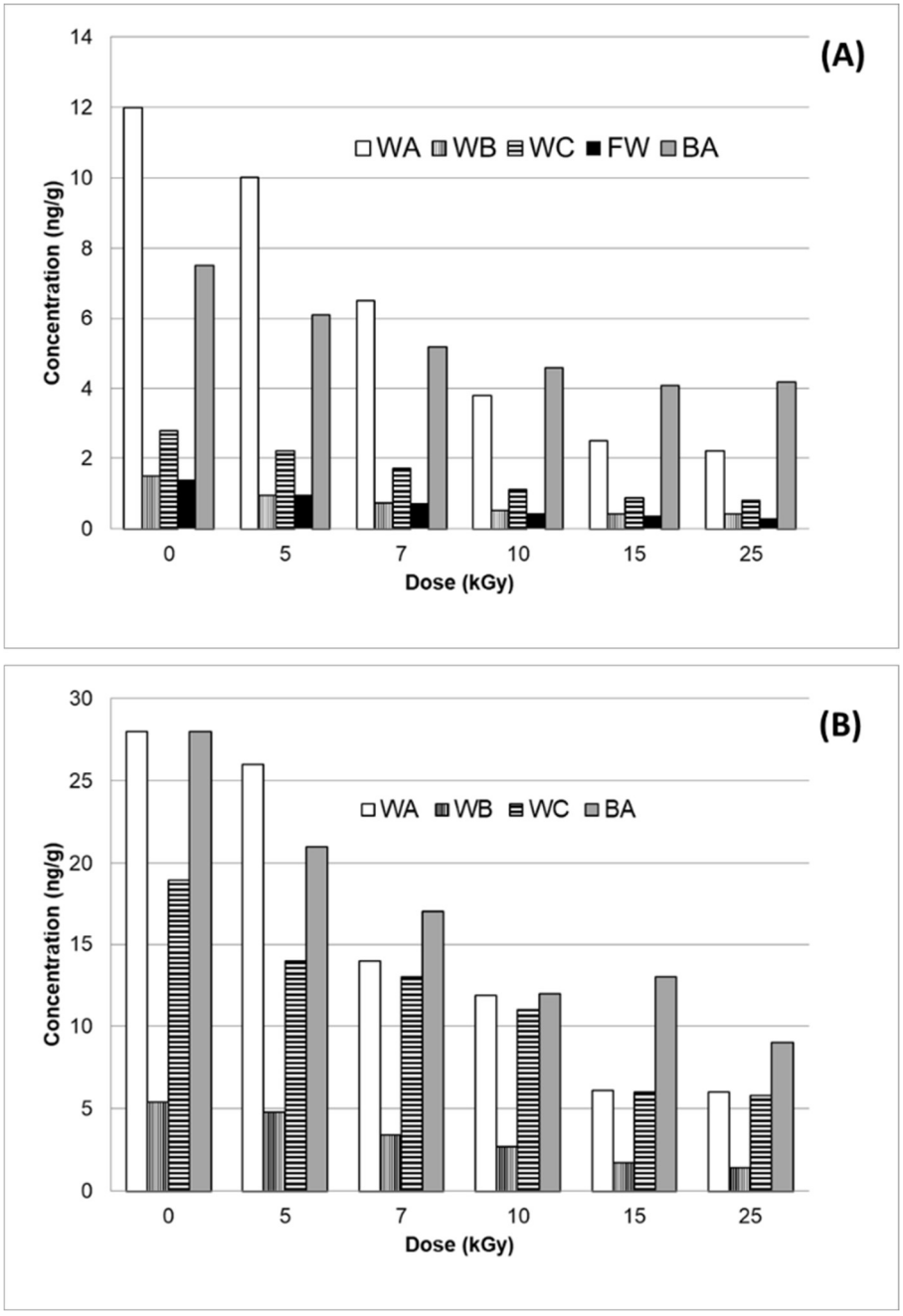

Figure 8. T-2 toxin (A) and HT-2 toxin (B) concentrations in the irradiated samples as dependence of the absorbed irradiation dose.

It can be determined that almost 50% of T-2 may be degraded at doses below 10 kGy, whereas later increase of dose dos not affect notable spread of structure susceptibility to radiation energy perhaps due to the complex trichothecene backbone structure. However, better degradation than that reported in the case of pure standards in ACN were noted in case of real grain samples.

Besides of these compounds, other T-2 class (Class A trichothecenes) were determined in the samples. The initial contents prior to irradiation and the contents and degradation rates of Class A at 10 kGy and 25 kGy doses are expressed in Table 6. Most of these compounds showed lower degradation rate as compared to that of T-2. The degradation rate of hydrolysed T-2 tetraol metabolites were notably lower perhaps due to potential formation of these metabolites during the radiolytic reactions, while further experiments are needed to confirm these assumptions.

Table 6. Degradation of irradiated Class A trichothecenes at 10 kGy and 25 kGy.

Matrix	Dose kGy	15-MAS	T-2*	HT-2	T-2TRI	T-2TETR	NEO
WA	0	2.4	12	28	<1	3.5	<0.25
	10	1.6	3.8	11.9	<1	2.1	<0.25
	25	1.1	2.2	6	<1	1.8	<0.25
	10 kGy efficiency	**33.3**	**68.3**	**57.5**	n/a	**40.0**	n/a
	25 kGy efficiency	**54.2**	**81.7**	**78.6**	n/a	**48.6**	n/a
WB	0	<1.1	1.5	5.4	<1	2.4	<0.25
	10	<1.1	0.52	2.7	<1	1.8	<0.25
	25	<1.1	0.42	1.4	<1	1.3	<0.25
	10 kGy efficiency	n/a	**65.3**	**50.0**	n/a	**25.0**	n/a
	25 kGy efficiency	n/a	**72.0**	**74.1**	n/a	**45.8**	n/a
WC	0	2.3	2.8	18.9	<1	1.9	<0.25
	10	1.7	1.1	11	<1	1.4	<0.25
	25	1.5	0.8	5.8	<1	1.3	<0.25
	10 kGy efficiency	n/a	**65.3**	**50.0**	n/a	**25.0**	n/a
	25 kGy efficiency	n/a	**72.0**	**74.1**	n/a	**45.8**	n/a
FW	0	<1.3	1.4	<3.1	<0.8	<0.5	<0.28
	10	<1.3	0.45	<3.1	<0.8	<0.5	<0.28
	25	<1.3	0.29	<3.1	<0.8	<0.5	<0.28
	10 kGy efficiency	n/a	**67.9**	n/a	n/a	n/a	n/a
	25 kGy efficiency	n/a	**79.3**	n/a	n/a	n/a	n/a
BA	0	13	7.5	28	1.9	18	0.69
	10	6.4	4.6	12	0.87	8.4	0.39
	25	4.3	4.2	9	0.69	5.5	0.3
	10 kGy efficiency	**50.8**	**38.7**	**57.1**	**54.2**	**53.3**	**43.5**
	25 kGy efficiency	**66.9**	**44.0**	**67.9**	**63.7**	**69.4**	**56.5**

DON is the major *Fusarium* mycotoxin regulated in cereals. It was determined that DON co-occurred with its derivatives (D3G, 3-AcDON, 15-AcDON) and NIV, which was the predominant mycotoxin of class B trichothecenes with highest contents determined in grain samples. It was determined in most cases that irradiation doses below 10 kGy did not affect notable degradation of class B trichothecenes. Increase of dose above 10 kGy was crucial for the degradation of DON and its derivatives.
Figure 9 shows the degradation of DON and NIV in different grain matrices as dependence of dose, whereas the effect of 10 and 25 kGy doses for all class B trichothecenes are summarised in Table 7.

It was determined that doses above 10 kGy are needed to increase efficiency on DON metabolite degradation.

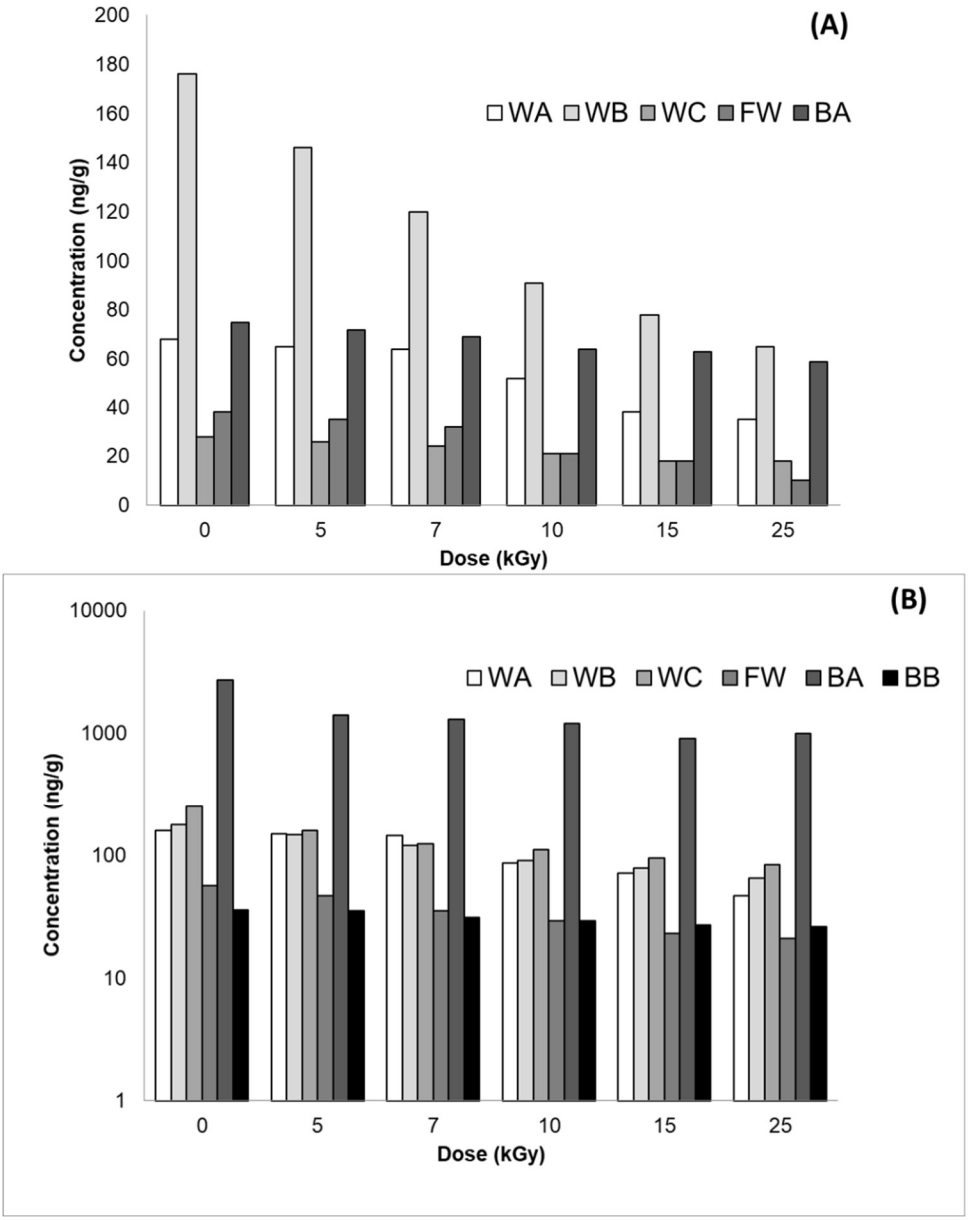

Figure 9. DON (A) and NIV (B) concentrations in the irradiated samples as dependence of the absorbed irradiation dose.

Table 7. Degradation of irradiated Class B trichothecenes at 10 kGy and 25 kGy.

Matrix	Dose kGy	NIV	DON*	D3G	3-AcDON	15-AcDON
WA	0	159	68	6.8	2.5	3.9
	10	86	52	6	1.9	3
	25	47	35	3.5	1.7	1.9
	10 kGy efficiency	**45.9**	**23.5**	**11.8**	**24.0**	**23.1**
	25 kGy efficiency	**70.4**	**48.5**	**48.5**	**32.0**	**51.3**
WB	0	176	85	82	<1.7	<2.5
	10	91	57	63	<1.7	<2.5
	25	65	37	30	<1.7	<2.5
	10 kGy efficiency	**48.3**	**32.9**	**23.2**	**n/a**	**n/a**
	25 kGy efficiency	**63.1**	**56.5**	**63.4**	**n/a**	**n/a**
WC	0	252	28	6.9	1	<2.5
	10	110	21	4.5	0.74	<2.5
	25	83	18	1.9	0.62	<2.5
	10 kGy efficiency	**56.3**	**25.0**	**34.8**	**26.0**	n/a
	25 kGy efficiency	**67.1**	**35.7**	**72.5**	**38.0**	n/a
FW	0	56	38	0.75	<2	<3.3
	10	29	21	0.31	<2	<3.3
	25	21	10	0.29	<2	<3.3
	10 kGy efficiency	**48.2**	**44.7**	**58.7**	n/a	n/a
	25 kGy efficiency	**62.5**	**73.7**	**61.3**	n/a	n/a
BA	0	2700	75	8.8	3.9	<3.3
	10	1200	64	6.4	2.9	<3.3
	25	1000	59	4.4	2.4	5.4
	10 kGy efficiency	**55.6**	**14.7**	**27.3**	**25.6**	n/a
	25 kGy efficiency	**63.0**	**21.3**	**50.0**	**38.5**	n/a
BB	0	36	<5.0	<1.7	<5	<3.6
	10	29	<5.0	<1.7	<5	<3.6
	25	26	<5.0	<1.7	<5	<3.6
	10 kGy efficiency	**19.4**	n/a	n/a	n/a	n/a
	25 kGy efficiency	**27.8**	n/a	n/a	n/a	n/a

Irradiated samples: stability of nonregulated *Fusarium* toxins (ENNs). No data of ionising radiation efficiency on ENNs was determined in previous studies. The naturally contaminated grain samples contained low to rather high contents of ENNs. ENN B was determined at rather high level (825 ng/g) in one of edible wheat samples. It was determined that evet at 5 kGy the level of ENN was

notably reduced and reached the maximum level of degradation around 60% after increase of the dose above 10 kGy.

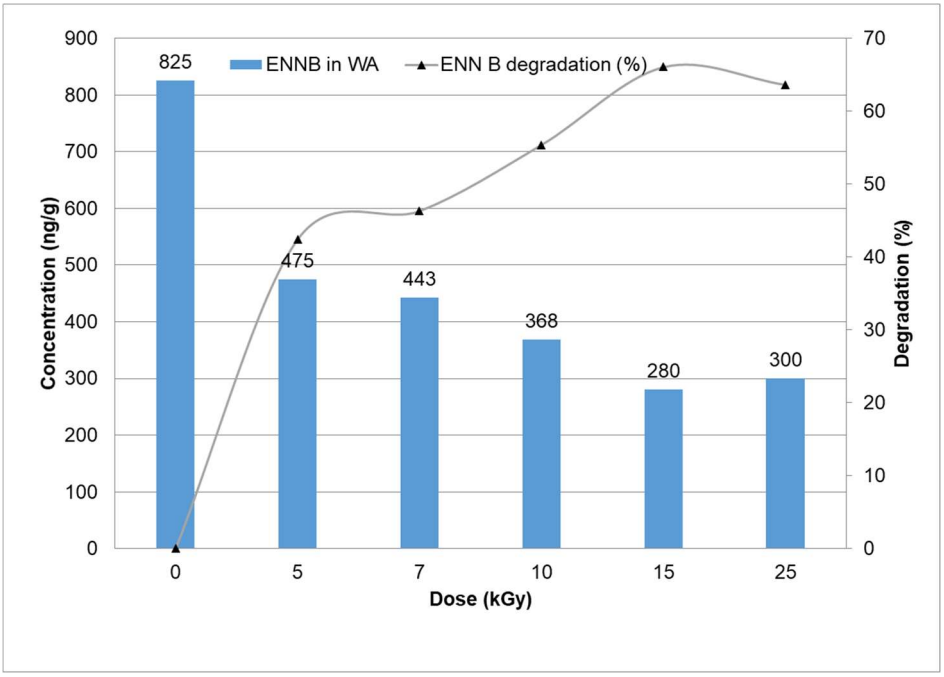

Figure 10. ENN B contents in wheat (WA) and characteristic degradation rate as dependence of the dose of irradiation.

Other two samples showed very low levels of ENN B and ENN B1 cooccurring in the barley (Figure 11). However, also in these samples it was determined that ENNs may be reduced notably starting with 5 kGy irradiation, whereas 10 kGy was the optimal dose for notable reduction of ENN contents.

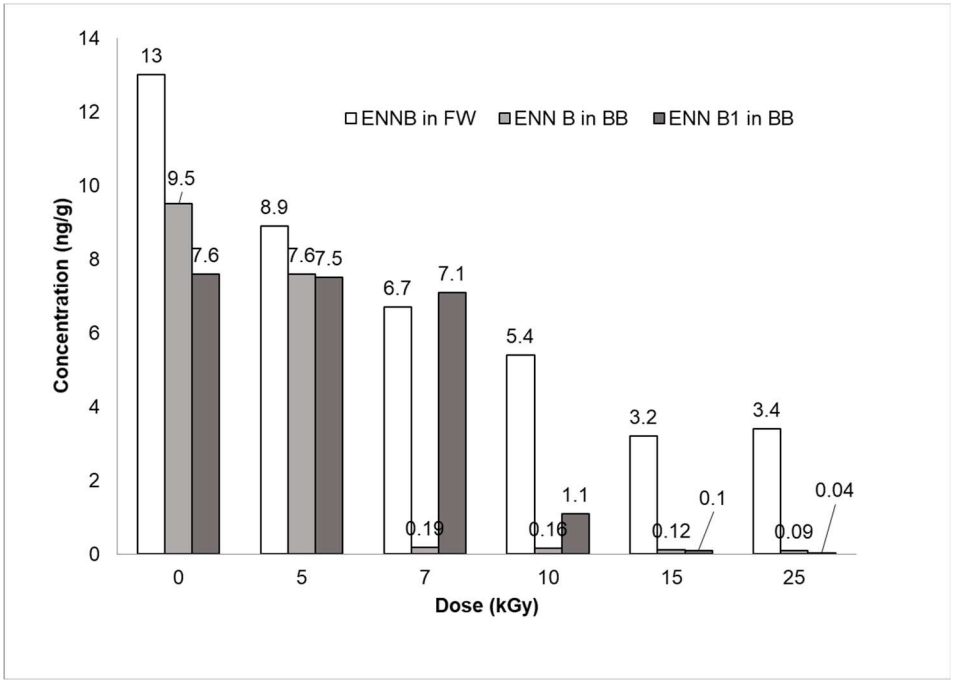

Figure 11. ENN B contents in FW and BB as dependence of the dose of irradiation.

Irradiated samples: stability of nonregulated *Alternaria* toxins. Alternaria toxins were determined at rather high frequency, but rather low contents, when comparing to trichothecenes (Table 8).
It was determined that complexity of matrices affected degradation of *Alternaria* toxins. From Fig. 12 it can be well seen that TEN levels were notably level in one of edible wheat samples compared to two other weat samples, whereas the data set is too small to make any considerations. Taking into account that TEN was the predominant of *Alternaria* toxins, the degradation of this compound was indicative and showed rather limited susceptibility to radiation at doses of 10 kGy, whereas increase of doses above 10 kGy was crucial to gain efficient decomposition of these toxins in grain matrices.

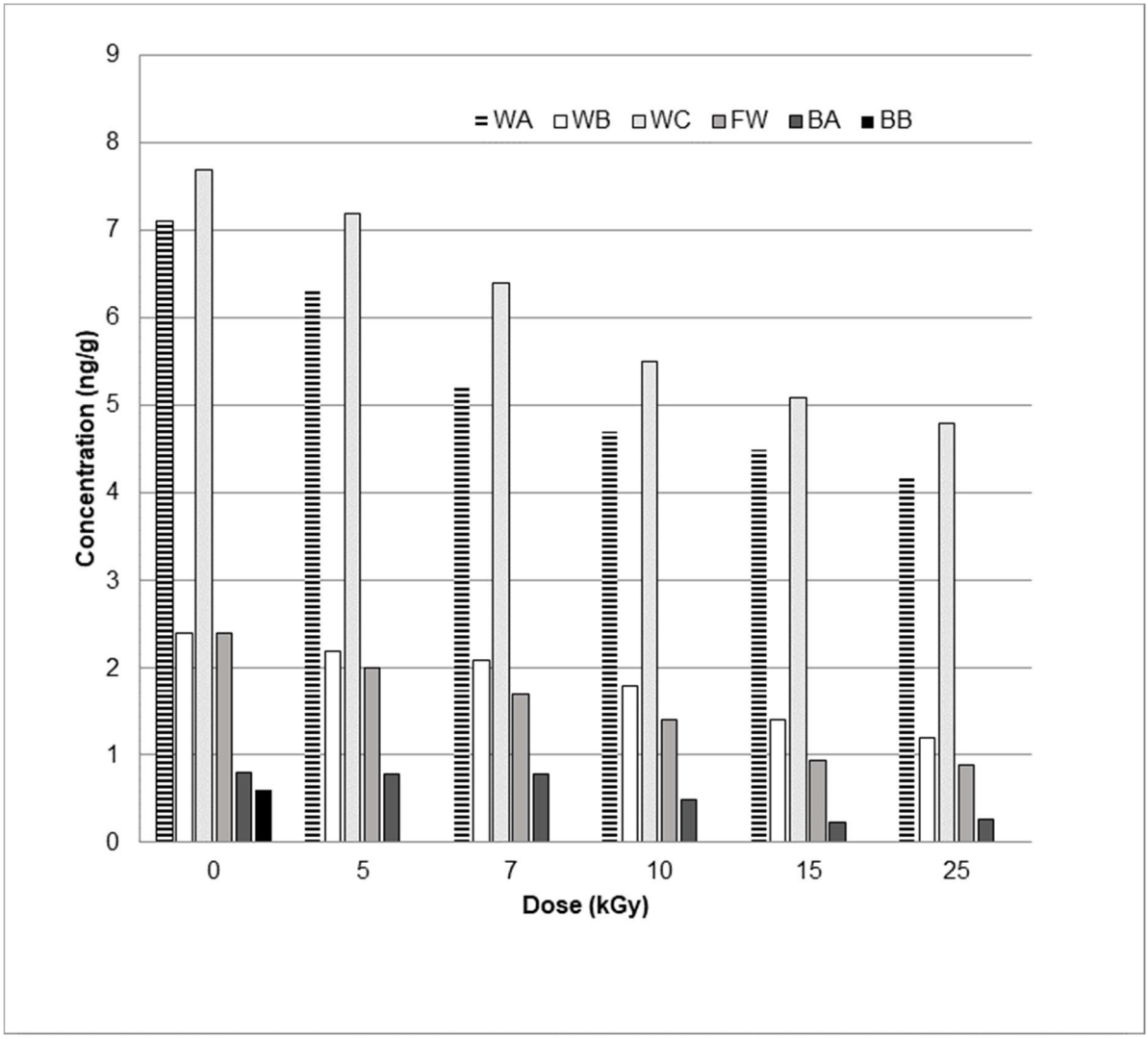

Figure 11. Tentoxin (TEN) contents in grain matrices and BB as dependence of the dose of irradiation.

It can be well seen also from the Table 8 that summarises data of other determined *Alternaria* toxins and their concentration levels prior and after irradiation up to 10 and 25 kGy.

Table 8. Degradation of irradiated Alternaria toxins at

Matrix	Dose kGy	TEN	ATX I	AOH	AME
WA	0	7.1	1.21	1.5	<1.4
	10	4.7	0.81	1.2	<1.4
	25	4.2	0.39	0.9	<1.4
	10 kGy efficiency	**33.8**	**33.1**	**20.0**	n/a
	25 kGy efficiency	**40.8**	**67.8**	**40.0**	n/a
WB	0	2.4	<0.36	4.3	<1.4
	10	1.8	<0.36	2.7	<1.4
	25	1.2	<0.36	2	<1.4
	10 kGy efficiency	**25.0**	n/a	**37.2**	n/a
	25 kGy efficiency	**50.0**	n/a	**53.5**	n/a
WC	0	7.7	1.4	1.8	3.8
	10	5.5	0.83	0.75	2.5
	25	4.8	0.45	0.51	1.8
	10 kGy efficiency	**28.6**	**40.7**	**58.3**	**34.2**
	25 kGy efficiency	**37.7**	**67.9**	**71.7**	**52.6**
FW	0	2.4	<0.63	<0.6	<2.5
	10	1.4	<0.63	<0.6	<2.5
	25	0.89	<0.63	<0.6	<2.5
	10 kGy efficiency	**41.7**	n/a	n/a	n/a
	25 kGy efficiency	**62.9**	n/a	n/a	n/a
BA	0	0.8	0.7	0.95	3.1
	10	0.49	0.56	0.6	2.1
	25	0.26	0.36	0.45	1.6
	10 kGy efficiency	**38.8**	**20.0**	**36.8**	**32.3**
	25 kGy efficiency	**67.5**	**48.6**	**52.6**	**48.4**
BB	0	0.6	<2.0	2.3	0.74
	10	<0.6	<2.0	<0.3	<0.08
	25	<0.6	<2.0	<0.3	<0.08
	10 kGy efficiency	n/a	n/a	**100.0**	**100.0**
	25 kGy efficiency	n/a	n/a	**100.0**	**100.0**

5. Conclusions

The effect of gamma irradiation (doses from 5 to 25 kGy) on wheat, barley and broad bean matrices was evaluated. The study of regulated mycotoxin standard solutions irradiated up to 10 and 25 kGy indicate challenges of mycotoxin degradation rate. The real sample analysis indicated notable role of the matrix type on the radiolytic processes affecting mycotoxin degradation.

Study of the control samples indicate to emerging mycotoxin (enniatins and *Alternaria* toxins) prevalence in grain and legume samples.

With few exceptions of ENNs in barley samples, most of the mycotoxins determined in wheat, barley and broad bean matrices showed rather high stability at low irradiation doses (below 10 kGy). Degradation rate ranged between 20 to 78%, when doses of radiation where increased to ≥10 kGy. This report for the first indicate data of emerging mycotoxin radiation stability in grain samples. Reported levels indicate of partial efficiency of gamma radiation on the degradation of these compounds. Further studies including comparison with other treatment methods and evaluation of secondary metabolite products are crucial to determine safety of ionising radiation application for mycotoxin management in food products.

The provided results show valuable insights for further evaluation of ionising radiation efficiency for the management of regulated or emerging mycotoxins in dry food matrices.

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