INFLUENCE OF DEGREE OF WHEAT INFESTATION WITH *Fusarium* ON ITS TECHNOLOGICAL QUALITY AND SAFETY

**ABSTRACT:** Climatic conditions prior to wheat harvest 2010 were favorable for the development of field molds. The most important wheat contamination that should be determined is the presence and frequency of grain lots infected with *Fusarium*. This paper presents the results of content of fusarious kernels found in samples collected from all wheat growing regions of Serbia. Investigations were performed according to the regulations which foresee sensory determination of content of infected kernels. Determined average content of fusarious kernels was 6.01%, varying in range from 0% to 29.65 %. The obtained results, which exceeded the upper limits of permitted contents defined by national and EU regulations, pointed to the need for investigation of presence of *Fusarium* metabolic products, i.e. mycotoxins (zearalenone (ZEA) and deoxinivalenol (DON)) in wheat. Mycotoxin content was determined in average wheat samples and in wheat samples with increased content of fusarious kernels. Direct enzymatic immunoaffinity test (ELISA) was applied for determination of mycotoxin content. Although high content of fusarious kernels characterizes wheat crop in 2010, the determined quantities of two investigated mycotoxins did not exceed maximal permitted content. Consequences of unfavorable quantity and structure of total impurities in wheat crop in 2010 reflect decreased commercial and technological quality of wheat and point out to the need of necessary wheat safety control.

**KEY WORDS:** *Fusarium* spp., quality, mycotoxins, wheat

INTRODUCTION

Based on general opinions and facts related to the occurrence of global climatic changes in the world, and especially Europe, problem of potential impact of climatic changes on food safety has become the topic of interest for many authors. Nevertheless, specific influence on food safety is still unresolved issue. The following climatic changes were identified as relevant for agriculture and food production: temperature increase, precipitation variations, drought and increased level of atmospheric CO₂. The extent of influence of potential favorable and unfavorable effects of climatic changes on cereal crops depends on the above mentioned factors (development of molds,
production of mycotoxins, pesticide residues, impurities and pathogen microorganisms) (Miraglia et al., 2009).

Climatic conditions in wheat growing regions of Serbia, in winter and autumn of 2009/2010, were favorable for germination and protection against winterkill. In the period of kernel forming and filling, rainfall above the average resulted in delay of wheat maturation, which caused postponed beginning of the harvest and generated conditions favorable for the development of molds and germination of wheat on the ear (http://www.hidmet.gov.rs/). In the period of ear formation, under favorable weather conditions (temperature and rainfall), wheat can be infected with molds from Fusarium genus (B r e r a et al., 1998).

All cereals differ regarding its sensitivity to Fusarium infestation and accumulation of mycotoxins (M a t t h i e s et al., 2000; S i n h a and S a v a r d, 1997; F o u r b e t et al., 2001). Infestation of wheat with Fusarium spp. (F. graminearum, F. culmorum, F. poae, F. avenaceum) results in yield decrease, but even greater threat represents food and feed safety risk due to mycotoxin contamination (M a t t h i e s et al., 2000). Molds form Fusarium genus have the ability to produce several hundred different mycotoxins characterized by different chemical and biological properties, and extremely different toxigenic activities (carcinogenic, genotoxic, teratogenic, hapatoxic, immunotoxic) (Z i - n a d i n e et al., 2006).

The aim of the research presented in this paper was to perceive the degree of infestation of wheat crop with Fusarium spp. in 2010, to analyze the influence of increased content of fusarious kernels on commercial and technological quality of wheat, and mycotoxin content in wheat samples.

MATERIALS AND METHODS

Commercial quality of 367 single lot wheat samples collected during the entire harvest from all wheat growing regions of Serbia was analyzed. Sampling was performed at 20 locations where harvested wheat was stored into warehouses. Composite samples were formed from single samples representing wheat from each of the involved locations, as well as the composite average wheat sample representing wheat harvested in 2010. On the basis of differences in protein and fusarious kernels contents, 13 additional composite wheat samples were formed. Commercial quality (test weight, protein content, gluten content, content and structure of impurities and Falling number) was determined for each sample, while technological quality (determination of dough properties using Brabender Farinograph, Extensograph and Amylograph) was determined for all composite samples. Fusarious and spoiled kernels were manually extracted from wheat harvested in 2010. For these samples, as well as for composite average sample of wheat harvested in 2010, quantitative determination of mycotoxins (aflatoxin (AF), ochratoxin A (OTA), zearalenone and deoxinivalenol) was conducted by direct enzymatic immunoaffinity method ELISA. Additional samples with high content of fusarious kernels were analyzed in respect to zearalenone and deoxinivalenol contents.
Moisture content, wet gluten content and sedimentation value of wheat were determined by Infratec 1241, according to the User Manual Infratec 1241 (Rev.2.3) – Foss Analytical AB, test weight was determined according to the method from the Regulation of methods of physical and chemical analysis for control of quality of wheat, milling and baking products and rapidly frozen dough (The Official Gazette SFRY 74/1988), Falling number was determined according to ICC standard No 107/1, while admixture content and structure was determined according to the Regulation about quality of agricultural products stored in public warehouses (The Official Gazette of Serbia 37/10). Determination of physical properties of wheat flour (dough) using Brabender Farinograph and Extensograph, and indirect determination of activity of cereal amylase using Brabender Amylograph were conducted according to ICC standards No. 115/1, No 114/1 and No. 126/1, respectively.

For the analysis of mycotoxins Veratox® Neogen aflatoksin (AF), ochratoxin A (OTA), zearalenone (ZEA) and deoxinivalenol (DON) test kits were applied. Readings of color intensity for quantification purposes was conducted at 650 nm using ELISA reader (Thermolabsystem, Thermo, Finland), and mycotoxin content was determined on the basis of calibration curve. According to producer’s instructions, detection limits for AF, OTA, ZEA and DON are 1, 2, 25 and 250 (μg/kg), respectively. Effects of different levels of fusarious kernel content on commercial and technological quality of wheat were analyzed according to ANOVA statistical procedure from Statistica 8.0 software.

RESULTS AND DISCUSSION

Climatic conditions during the production period for wheat crop in 2010 were favorable for the development of field molds. On the basis of frequency analysis of contents of fusarious kernels determined in the samples of wheat crop in 2010, the distribution curve of fusarious kernels content was constructed (Figure 1).

Distribution curve of fusarious kernels content in wheat crop in 2010 has the shape of Gamma distribution with 6 degrees of freedom. The largest share of samples belongs to the interval of up to 10% of fusarious kernels in the sample, with the largest frequency of samples having relatively high content of fusarious kernels, from 2 to 4%. Frequency of samples with more than 10% of fusarious kernels gradually decreases towards higher contents of fusarious kernels, but samples with even more than 20% of fusarious kernels, although with low frequencies, were registered. Presented data confirm the assumption that wheat crop in 2010 had risky high content of fusarious kernels (Figure 2).

Samples with fusarious kernel content from 1.01 to 9.00% had the highest share (79%), 51% of samples of wheat crop in 2010 was with fusarious kernel content from 1.01 to 5.00%, and 28% of samples with fusarious kernels content from 5.01 to 9.00% (Figure 2). Only 4% of analyzed samples were characterized with fusarious kernel content under acceptable 1%. High share of samples with high fusarious kernel content points to the possible risk of mycotoxin
Fig. 1 – Distribution curve of fusarious kernels content in wheat crop in 2010

Fig. 2 – Share of samples of wheat crop from 2010 (%) depending on the range of fusarious kernel content
occurrence, as well as to the possible consequences of presence of high content of fusarious kernels that could influence commercial and technological quality of wheat.

In order to perceive the consequences of presence of different contents of fusarious kernels on commercial quality of wheat, expressed on the basis of test weight, protein content, wet gluten content, sedimentation value, falling number, content and structure of impurities, average values for samples grouped on the basis of level of fusarious kernels is presented in Table 1.

Tab. 1 – Influence of level of content of fusarious kernels on average commercial quality of wheat crop in 2010

<table>
<thead>
<tr>
<th>Range of fusarious kernels content (%)</th>
<th>&lt; 1</th>
<th>1,01-5,00</th>
<th>5,01-9,00</th>
<th>9,01-13,00</th>
<th>13,01-17,00</th>
<th>&gt; 17,00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>14</td>
<td>189</td>
<td>103</td>
<td>25</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Parameters</td>
<td></td>
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<tr>
<td>Fusarious kernels content (%)</td>
<td>0,45^a</td>
<td>3,25^b</td>
<td>6,82^c</td>
<td>10,18^d</td>
<td>14,40^e</td>
<td>22,70^f</td>
</tr>
<tr>
<td>Test weight (kg/m³)</td>
<td>755,6^a</td>
<td>749,5^ab</td>
<td>737,4^b</td>
<td>735,6^b</td>
<td>736,2^b</td>
<td>739,2^b</td>
</tr>
<tr>
<td>Protein content (%)</td>
<td>13,28^a</td>
<td>13,06^a</td>
<td>13,24^a</td>
<td>12,79^ab</td>
<td>12,30^b</td>
<td>12,82^ab</td>
</tr>
<tr>
<td>Wet gluten content (%)</td>
<td>26,39^a</td>
<td>25,65^ab</td>
<td>25,88^ab</td>
<td>24,75^bc</td>
<td>23,74^c</td>
<td>24,47^bc</td>
</tr>
<tr>
<td>Sedimentation value</td>
<td>41^a</td>
<td>39^a</td>
<td>39^a</td>
<td>37^ab</td>
<td>34^b</td>
<td>37^ab</td>
</tr>
<tr>
<td>Falling number according to Hagberg (s)</td>
<td>238^a</td>
<td>249^a</td>
<td>228^a</td>
<td>243^a</td>
<td>253^a</td>
<td>271^a</td>
</tr>
<tr>
<td>Total removable impurities content (%)</td>
<td>2,08^a</td>
<td>2,68^ab</td>
<td>2,94^bc</td>
<td>3,05^bc</td>
<td>3,67^c</td>
<td>3,20^bc</td>
</tr>
<tr>
<td>Total difficultly removable impurities content (%)</td>
<td>5,95^a</td>
<td>9,12^b</td>
<td>13,26^c</td>
<td>17,50^d</td>
<td>21,05^c</td>
<td>28,54^f</td>
</tr>
<tr>
<td>Total impurities content (%)</td>
<td>8,03^a</td>
<td>11,80^b</td>
<td>16,20^c</td>
<td>20,55^d</td>
<td>24,71^c</td>
<td>31,74^f</td>
</tr>
</tbody>
</table>

ANOVA statistical procedure and Duncan’s test were applied for testing the significance of differences among average values of selected commercial quality parameters for samples (lots) of wheat with different level of fusarious kernel content (Table 1). Blends of wheat in which content of fusarious kernels was under 1.00%, were characterized with statistically different test weight in comparison to wheat blends with higher content of fusarious kernels. Wheat blends with content of fusarious kernels between 1.01 to 5.00% were characterized with test weight which differed from wheat with higher content of fusarious kernels, but the difference was not statistically significant.

When content of fusarious kernels in wheat is related to protein content, two groups of samples can be identified. In the first group there are wheat blends with fusarious kernel content of up to 9.00%, while the second group comprises of wheat blends with fusarious kernel content over 9.00%. The second group of samples is opposite to the findings of numerous authors (Boyaçioğlu and Hettiarachchy 1995; Jackowiak, et al., 2005) and is characterized with lower protein content. This result was probably the consequence of the fact that wheat samples with fusarious content, originating
from all wheat growing regions of Serbia and representing different wheat varieties, were grown under different climatic conditions, and during the production different agricultural treatments were applied. The same effect could be observed in the case of sedimentation value.

Decrease trend of wet gluten content with an increase of fusarious kernel content could be noticed, but differences were not statistically significant.

Fusarious kernel content did not have statistically significant influence on Hagberg falling number, but the trend of increase of both falling number and fusarious kernels (above 9%) was noticed. It is well known that wheat varieties and growing location can significantly influence the falling number values. Nitrogen fertilization slightly increases falling number. According to the results of Wang (2004), wheat infected with *Fusarium culmorum* is characterized with higher falling number values, indicating that falling number is not sufficiently suitable method for determination of alpha amylase activity in flours contaminated with molds.

Analysis of influence of fusarious kernel content on total content of removable impurities showed no statistically significant differences, but the trend of increase of total content of removable impurities with increase of fusarious kernel content existed. The removable impurities are composed mainly of shrunken and broken kernels which pass through 2 mm screening sieve. Wheat kernels infected with *Fusarium* in early kernel formation phases are without any doubt shrunken and can be found in removable impurities. When shrunken kernels are considered, although they cannot be directly connected to removable impurities, an indirect result is the registration of increase of removable impurities content.

Wheat blends with different levels of fusarious content differ with respect to the level of difficulty of impurity removal. Increased content of fusarious kernels in formed wheat blends results in difficult impurity removal. Fusarious kernels are considered as impurities difficult to for removal, which jeopardizes technological quality and safety of wheat due to inability of separation, resulting in transition of fusarious kernels to further processing steps. The same trend can be noticed in the case of total impurities which is higher than the tolerated limit of 8%, mainly as the consequence of impurities that are highly difficult to remove, among which fusarious kernels are predominant.

In order to comprehend the influence of fusarious kernel content in wheat blends on wheat technological quality parameters, composite wheat samples, in which rheological properties were analyzed, were grouped with respect to fusarious kernel content (Table 2).

Most of the average values of analyzed wheat, among which samples characterized with different content of fusarious kernels were present, showed no statistically significant differences in technological quality parameters. Increase of content of fusarious kernels is accompanied with trends of decrease in Farinograph quality number, dough stability and resistance, and trends of increase in dough degree of softening, Extensograph energy, resistance and ratio number (Table 2). Statistically significant differences were confirmed only in the case of Amylograph peak viscosity without confirmation based on
Falling number value (Table 1), but in accordance with the findings of some authors, Wang et al. (2005), who discovered that the samples with extremely high content of fusarious kernels were characterized with obviously higher values of Hagberg Falling number, whereas saccharose content in flour decreased. Other authors stated that contamination of wheat with storage molds (Aspergillus spp. and Penicillium spp.) resulted in decrease of baking performance of wheat flour. It is interesting to mention that the content of mycotoxins (DON) in analyzed wheat samples was not in relation with rheological parameters and flour baking properties (Antes et al., 2001).

Fungal amylolytic decomposition leads to degradation of small starch granules (Jacakowski et al., 2005), and due to decreased total specific area of starch granules and higher degree of damage of starch component of endosperm, in comparison to healthy kernels, fusarious kernel becomes more subjected to decomposition of starch component by fungal alpha amylase. The consequence of this phenomenon is the decrease in Amylograph peak viscosity.

Boyaçioğlu and Hettiarachchy (1995) confirmed that in wheat, moderately contaminated with F. graminearum, albumin content decreased by 33% and glutenine content by 88%, in comparison to the control sample, in spite of higher total protein content in the contaminated samples than in the healthy kernels. Meyer et al. (1986) found out that German varieties contaminated with F. culmorum had worse baking properties which attributed to damaged gluten fractions. Dexter et al. (1996) determined that red spring wheat contaminated with Fusarium resulted in altered flour color, weak dough properties and unsatisfactory bread quality. Gluten originating from fusarious wheat had lower content of glutenin fraction in comparison to gluten of healthy wheat.

<table>
<thead>
<tr>
<th>Range of fusarious kernels content (%)</th>
<th>≤ 5,00</th>
<th>5,01-10,00</th>
<th>≥ 10,00</th>
</tr>
</thead>
<tbody>
<tr>
<td>broj uzoraka</td>
<td>6</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amzlograph peak viscosity (Au)</td>
<td>248a</td>
<td>252a</td>
<td>174b</td>
</tr>
<tr>
<td>Faringraph water absorption (%)</td>
<td>56,8a</td>
<td>56,6a</td>
<td>57,5a</td>
</tr>
<tr>
<td>Faringraph dough development time (min)</td>
<td>2,2a</td>
<td>2,0a</td>
<td>2,0a</td>
</tr>
<tr>
<td>Faringraph dough stability time (min)</td>
<td>1,5a</td>
<td>1,1a</td>
<td>0,8a</td>
</tr>
<tr>
<td>Faringraph dough mixing resistance (min)</td>
<td>3,7a</td>
<td>3,2a</td>
<td>2,8a</td>
</tr>
<tr>
<td>Faringraph dough degree of softening (Fu)</td>
<td>96a</td>
<td>98a</td>
<td>110a</td>
</tr>
<tr>
<td>Faringraph quality number</td>
<td>55,8a</td>
<td>53,8a</td>
<td>51,3a</td>
</tr>
<tr>
<td>Extensograph energy (cm²)</td>
<td>31a</td>
<td>32a</td>
<td>34a</td>
</tr>
<tr>
<td>Extensograph dough resistance at 5 cm (Eu)</td>
<td>111a</td>
<td>121a</td>
<td>128a</td>
</tr>
<tr>
<td>Extensograph dough extensibility (mm)</td>
<td>159a</td>
<td>153a</td>
<td>155a</td>
</tr>
<tr>
<td>Extensograph ratio</td>
<td>0,68a</td>
<td>0,79a</td>
<td>0,83a</td>
</tr>
</tbody>
</table>
Determined content of fusarious kernels in wheat blends, formed on the basis of different ranges of protein content as the wheat segregation parameter applied by relatively small number of warehouses that segregate wheat in Serbia, is presented in Figure 3.

![Fig. 3 – Content of fusarious kernels (%) in wheat blends formed from wheat samples from different protein content ranges](image)

The highest content of fusarious kernels was determined in wheat samples with protein content above 14%\textsubscript{dmb}. This result is in concordance with the findings of many authors (Boyacioglu and Hetiarachchy 1995; Jackowiak et al., 2005). Higher fusarious kernels content was also determined in wheat blend obtained from samples with protein content under 11.5%\textsubscript{dmb}. This is probably the consequence of influence of set of factors: soil composition, climatic conditions, inappropriate agricultural measures, absence of crop protection and other.

In order to obtain preliminary insight into presence of mycotoxins in wheat crop from 2010, the investigations of mycotoxin presence in the selected samples were conducted as presented in Table 3.

In average wheat sample from crop 2010 and in sample No.3 (5.65% of fusarious kernels), in spite of high fusarious kernel content (6.01%), the presence of mycotoxins AF, OTA, ZEA was not determined. Determined content of DON was under permitted limits according to the European regulations (1250 µg/kg). In the samples of fusarious kernel, content of mycotoxin ZEA was beyond both national (1 µg/kg) and European (100 µg/kg) regulations. Content of DON was determined to be above the limit of quantification of applied...
method and above European regulations (1250 μm/kg) in the same samples. In the sample of spoiled kernels, as well as in the sample No. 2 (8.83% of fusarious kernels) determined contents of ZEA and DON were above the limits of European regulations. Having in mind the possibility of false positive results which can be obtained by applying ELISA method, it is important to check the obtained results with a confirmative method. Guy et al. (2004) proved that dark bread obtained from wheat after cleaning and intensive scrubbing contains about 40-50% of starting quantities of OTA, independently of level of starting contamination of wheat. Starting contamination of 20-30% was determined in white bread. There are not many results concerning the content of DON in products of wheat processing. DON is stable and results in contamination of corn even under extreme pH values and temperature. As for ZEA, it was determined that 60% of starting contamination remained in bread and 50% in pasta.

Results from multiple research confirm that fungal breeds of the same species, even their isolates from the same region, express variability in mycotoxin production (Blaney and Domman, 2002; Lorens et al., 2004b; Vogelsang et al., 2008a; Kokkonen et al., 2010). All presented facts point at actual risk of presence of mycotoxins (ZEA, DON) in wheat crop from 2010 in the quantities close to limits permitted by European and national regulations.

**CONCLUSIONS**

Wheat crop from 2010 is characterized with high content of fusarious kernels. Most samples are characterized with fusarious kernel content at the level of 1 to 9%. Only 4% of samples had content of fusarious kernels that was under 1%, content permitted by the regulations.
Increased content of fusarious kernels resulted in decrease of commercial (decreased test weight and protein content and increased content of impurities) and technological quality (trends of decrease of Farinograph quality number, stability and resistance of dough, Amilograph peak viscosity and trend of increase of Farinograph dough degree of softening) of wheat.

Increased content of fusarious kernels in wheat crop from 2010 resulted in increased risk of occurrence of mycotoxins in wheat and consequently in wheat products. On one hand, this emphasizes the need of application of agricultural measures for prevention of development of fusarium in agricultural production, while on the other hand, it also points to the need for introduction of contemporary wheat cleaning methods which enable separation of fusarious kernels from wheat prior to processing.

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УТИЦАЈ СТЕПЕНА ЗАРАЖЕНОСТИ ПШЕНИЦЕ МИКОТОКСИНОМ FUSARIUM НА ЊЕН ТЕХНОЛОШКИ КВАЛТИТЕТ И ИСПРАВНОСТ

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Резиме

Климатски услови пред жетву 2010. године били су повољни за развој плеśni. Најважнији аспект код загађења пшенице је одређивање присуства и учесталости микотоксина из рода Fusarium на парцелама пшенице. У раду су представљени резултати истраживања количине фузариозних зrna у узорцима узетим из делова Србије у којима се узгаја ова житарица. Истраживање је обављено у складу са прописима који предвиђају одређивање садржаја заражених зrna. Утврђена количина фузариозних зrna је у просеку била 6,01%, варирајући од 0% до 29,65%. Добијени резултати су прелазили горње дозвољене границе које су утврђене европским регулативима, што указује на неопходност даљег истраживања метаболичких производа који су заражени врстама из рода Fusarium, тј. микотоксинима (зеараленоне ZEA) и деоксиниваленол (DON)). Садржај микотоксина је установљен у узорцима пшенице просечних карактеристика, као и у узорцима пшенице са повећаним садржајем фузариозних зrna. Садржај микотоксина је утврђен помоћу ELISA теста. Јако усев пшенице из 2010. године има присутан велики броj фузариозних зrna, утврђене количине два испитивана микотоксина нису прелазиле максимално дозвољену границу. Последица неповољне количине и структуре нечистоћа које су утврђене у пшеници из 2010. године је смањен тржишни и технолошки квалитет пшенице, што указује на неопходну контролу квалитета ове житарице.