WHEAT SAFETY IN RELATION TO PRESENCE AND CONTENT OF DEOXYNIVALENOL

ABSTRACT: HACCP (Hazard Analysis and Critical Control Point) is a concept which identifies, evaluates, and controls hazards, significant for food safety. It is applicable to the entire food chain, “from farm to table”. Prevention of a foodborne mycotoxin contamination of commodities is the most rational and cost-effective method for preventing adverse effects of fungal metabolites on human and animal health. Deoxynivalenol (DON) belongs to the group of mycotoxins produced by certain Fusarium species, which can damage several vital organs, or demonstrate immunotoxic effect, when ingested in small amounts for a longer period of time. Of particular concern is exposure of children to this mycotoxin through cereals, which are believed to lead to reduced weight gain and decreased liver weights. For that reason, we tried to present HACCP concept for preventing wheat contamination with deoxynivalenol. To be able to apply this system, hazards must be identified, and risks assessed, and for that purpose a real picture of area (region) in which preventive measures shall be applied, need to be established. According to the results of the study conducted in the laboratory at our department, DON contaminated wheat samples in the region of Vojvodina accounted for 41.6% in 2004 and 2005, whereas amounts of deoxynivalenol ranged from 57 to 1840 μg/kg.

KEY WORDS: HACCP, deoxynivalenol, wheat

INTRODUCTION

HACCP system itself consists of two fundamental components: HA and CCP. HA is a risk analysis, that is, identification of potential hazards in each cycle of food production, and assessment of their effects on human health. CCPs (critical control points) are actions introduced throughout the production process by which food safety risks can be prevented, eliminated or reduced to acceptable levels.

Application of HACCP system is compatible with the quality management system, being in compliance with ISO 9000 series of standards, and it
implies adoption of appropriate measures required for food safety management. Prior to introduction of HACCP system in any organizational part of the food manufacturing establishment, the relevant organizational part must function in compliance with the principles of GAP (Good Agricultural Practice), GMP (Good Manufacturing Practice), GHP (Good Hygiene Practice) and relevant food safety legislation. For the purpose of avoiding any food-related risks, HACCP method is to be used as an effective way of managing and controlling food safety hazards in the preparation and handling of food and food products.

**HACCP INTRODUCTION PROCEDURE**

- Assemble of HACCP team
- Description of the product
- Identification of the intended product use

Since HACCP system involves multidisciplinary approach, multidisciplinary team should be assembled, consisting of specialists with a thorough knowledge of the product, production process, management and quality assurance principles. This team could include personnel from the production/sanitation, quality control, laboratory, engineering and independent outside experts to advise about the identified issues, or problematic areas. The task of the team is to ensure that all conditions for the application of the HACCP principles have been met and necessary documentation prepared, and the team members, therefore, must have the knowledge and expertise to develop an HACCP plan.

The product and production process description should include the name of the product, ingredients and composition, packaging, shelf life, storage conditions, and distribution control measures, if applicable.

The intended use of the product refers to its normal use by end-users, or consumers. The HACCP team must specify where the product will be sold, as well as the target group and labeling instructions which should be followed.

In our case, control is aimed at deoxynivalenol (DON), a frequent contaminant of cereals. The global frequency of mycotoxin-contamination of food appear to be increasing in the recent years. Mycotoxins may occur during the different stages of grains production. *Fusarium* species can be formed in the field preharvest, and are usually referred to as “field mycotoxins”, and may continue to be formed in storage if conditions are favorable.

*Fusarium* is one of the most widespread genera of plant pathogenic fungi responsible for destructive and economically very important diseases of cereal crops. *Fusarium* strains are responsible for wilts, rots and the like in legumes, coffee, pines, wheat, corn, carnation and grasses.

Disease of wheat known as Fusarium head blight (FHB) is caused primarily by *Fusarium graminearum* species. This disease reduces yield and seed quality grade and spreads easily, particularly in regions with humid climatic conditions (Bowden et al., 2003).

Symptoms: FHB is detected immediately after blooming. Infected heads become whitish, whereas leaves and stalks remain unchanged. A portion of
stalk immediately below the head is sometimes of chocolate brown colour. In-
fected wheat seeds are shrunken and typically chalky white or pinkish. Seeds
above the infected spikelet will shrunk if the mould enters the glume and dis-
rupts food supply. Apart from reducing yield and grade, this disease may also
contaminate the grain with fungal toxins (mycotoxins). FHB is favoured by
wet conditions during the flowering and early stages of kernel development.
FHB is recognized in the field by premature bleaching of infected spikelet,
and the production of orange, spore-bearing structures (S e r a f i n c h o n, 2003).
During wet weather, there may be whitish, occasionally pinkish, fluffy fungal
growth on infected heads in the field. Diseased spikelet can contain visibly af-
fected kernels. The grading term given to visibly affected wheat seeds is fusa-
rium damaged kernel (FDK).

The last line of defense occurs when *F. graminearum* infections are iden-
tified in cereal fields. Such occurrence would trigger an eradication strategy
where the crop is immediately ensiled or harvested, and the residue carefully
managed and cultivated. The field would be taken out of cereal production for
three years to allow the crop residue to degrade, resulting in the eradication of
the pathogen. *F. graminearum* does not survive in fields where the host mate-
rial has degraded and is no longer present. During the growing season, grow-
ers should check the fields for the presence of this disease. They should check
for symptoms on cereal heads, about three weeks after anthesis, and in the
grain at harvest. If *F. graminearum* is found, the planting of varieties least su-
sceptible to the disease should be considered.

Mycotoxins produced by *Fusarium* species significantly differ in their
structure, and the toxin production is primarily due to genetic factors, but is also
affected by the conditions in which fungi grow, such as: the amount of avail-
able water in substrate, for fungi growth (a_w), ambient temperature, presence
of aerobic medium, relative air moisture, type of medium or substrate, pH en-
vIRONMENT, level of sustained damage on kernels, total number of fungi and
share of toxicogenic species, as well as the presence of competitive microflora
etc. For moulds growth, humidity over 12%, and water activity above (a_w) 0,7,
is needed. Temperature favourable for toxin production ranges from −5 to
+60°C, and pH from 5—7.

*Fusarium* is a large and complex species containing strains adapted to dif-
ferent environmental conditions all over the world.

Different strains from the same species may behave differently in myco-
toxin production. There is also a regional difference in mycotoxins produced
by *Fusarium* species, as a result of genetic variation and climatic factors.
Generally speaking, it is not possible to determine series of conditions or fac-
tors having critical effect on the moulds growth and mycotoxin production. It
is a distinctive feature of biosynthesis of secondary metabolites; the produced
amount is conditioned not only by the environmental and nutritional parame-
ters, prevailing during the production, but is also affected by the previous crop
residues and the incidence of mould occurrence. Mycotoxin type and concen-
tration is always determined by the mould, substrate and external factors.
External factors affecting mould production can be physical, chemical and bio-
logical, but almost always in interaction with each other. The sheer fact that
Mycotoxins are able to synthesize in a few days under favourable ambient conditions, suggests that mycotoxin hazards are permanently present (Narech, 2006).

Trichothecenes are secondary toxic products produced by several moulds, including species of the genera Fusarium, Myrothecium, Trichoderma, Trichothecium, Stachybotrys and Verticimonsporium. More than 170 trichothecenes have been isolated and chemically characterized up to now. Trichothecenes are tetracyclic, sesquiterpenoid compounds with a 12,13-epoxy-group. Epoxide group C12,13 of all trichothecenes is believed to be responsible for their toxicity. Based on the presence of characteristic functional groups, they are divided into type A (T-2 toxin, diacetoxyscirpenol), type B (deoxynivalenol, nivalenol), type C and D. Trichothecenes from groups A and B are probably the most widely distributed mycotoxins, and according to the Report of the EU Commission, trichothecene toxins from group B are more prevalent in grains than those from group A. The most common trichothecene from group B is deoxynivalenol (DON) (57%), followed by 15-acetylenivalenol (20%), nivalenol (16%), fusarenon X (10%), and 3-acetylenivalenol (8%). Out of trichothecenes from group A, the most common is T-2 toxin (20% of analyzed samples), followed, to a lesser extent, by other toxins, namely, HT-2 toxin (14%), T-2 triol (6%), DAS (4%), MAS (1%), and verrucarol, but only in one out of 121 analyzed samples (SCOOP, 20).

F. graminearum and F. culmorum. are fungi most often associated with deoxynivalenol production have Presence of this moulds is prevalent in grains, wheat and corn, in particular, causing their rotting. They can also occur on barley, oats and rye, as well as grain products-malt, beer and bread (Scientific Committee on Food, 1999).

Hot and moist climate favours wheat infection with these moulds, taking place throughout the entire blooming phase. The optimum temperature for F. graminearum growth is found to be 25°C, annual % of humidity, and for F. culmorum 21°C, and 87% of humidity. According to Martins and Martins (2002) the most favourable conditions for DON production are 22°C (6.0 mg/kg) and 28°C (5.5 mg/kg), after 35 days of incubation. According to the same authors, there is no DON production at temperature of 37°C.

The presence of DON and other Fusarium mycotoxins in Europe is of a particular importance, primarily due to climatic conditions prevailing in that region. The Study of scientific corporation for food issues — sub-commission for trichothecene (SCOOP, 2003) included 11 EU states. Of 11.022 analyzed samples, DON presence was found in 57%. In a huge number of wheat and wheat flour analyzed for DON presence (6358), 61% of the samples were found to be positive, in the detection range from 2 µg/kg (Sweden) to 50 000 µg/kg (France).

The EU Scientific Committee for Food (Commission Regulation No 856/2005) has established maximum tolerable levels for DON in cereals and their products: unprocessed cereals other than durum wheat, oats and maize (1250 µg/kg), unprocessed durum wheat and oats (1750 µg/kg), unprocessed maize (1750 µg/kg), cereal flour, including maize flour, maize grits and maize meal (750 µg/kg), bread, pastries, biscuits, cereal snacks and breakfast cereals (500
μg/kg), pasta (dry, 750 μg/kg), processed cereal based food for infants and young children, and baby food (200 μg/kg).

Peters et al. (2001) calculated a provisional TDI of 1,1 μg/kg of body according to the results of the study conducted in the Netherlands. Based on this limit, they proposed a concentration limit of 129 μg DON/kg for wheat, a level designed to protect the health of children who are heavy consumers of wheat based products. This team of scientists concluded that in the 1998—2000 period (high amounts of DON were established in wheat) DON intake exceeded provisional TDI, particularly in children, which might have adverse effects on some children. In part because of the concerns raised in their studies, even stricter limits on DON exposures in bread have been established by the Dutch government, namely, 120 DON μg/kg BW for processed wheat and wheat-based products with wheat content of 33%, and 60 μg/kg.

Results of wheat samples analyzed for the presence and content of DON in the laboratory at the Department for Livestock Breeding at the Faculty of Agriculture in Novi Sad are shown in Table 2.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Total no. of samples</th>
<th>% of positive</th>
<th>Max. DON content</th>
<th>Average DON content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>1999</td>
<td>68</td>
<td>57</td>
<td>1250</td>
<td>310</td>
</tr>
<tr>
<td>Austria</td>
<td>2000</td>
<td>62</td>
<td>61</td>
<td>6090</td>
<td>1203</td>
</tr>
<tr>
<td>Austria</td>
<td>2001</td>
<td>36</td>
<td>50</td>
<td>1230</td>
<td>334</td>
</tr>
<tr>
<td>Belgium</td>
<td>2001</td>
<td>33</td>
<td>15</td>
<td>504</td>
<td>343</td>
</tr>
<tr>
<td>Germany</td>
<td>1999</td>
<td>26</td>
<td>80</td>
<td>764</td>
<td>285</td>
</tr>
<tr>
<td>Germany</td>
<td>2000</td>
<td>27</td>
<td>66</td>
<td>402</td>
<td>159</td>
</tr>
<tr>
<td>Denmark</td>
<td>1999</td>
<td>16</td>
<td>87</td>
<td>527</td>
<td>198</td>
</tr>
<tr>
<td>Denmark</td>
<td>2000</td>
<td>28</td>
<td>92</td>
<td>330</td>
<td>63</td>
</tr>
<tr>
<td>Denmark</td>
<td>2001</td>
<td>30</td>
<td>63</td>
<td>204</td>
<td>56</td>
</tr>
<tr>
<td>Finland</td>
<td>1999</td>
<td>37</td>
<td>67</td>
<td>264</td>
<td>88</td>
</tr>
<tr>
<td>Finland</td>
<td>2000</td>
<td>35</td>
<td>71</td>
<td>1026</td>
<td>234</td>
</tr>
<tr>
<td>Finland</td>
<td>2001</td>
<td>39</td>
<td>35</td>
<td>376</td>
<td>90</td>
</tr>
<tr>
<td>France</td>
<td>2001</td>
<td>53</td>
<td>100</td>
<td>2125</td>
<td>190</td>
</tr>
<tr>
<td>France</td>
<td>2002</td>
<td>3</td>
<td>33</td>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td>Norway</td>
<td>2001</td>
<td>147</td>
<td>34</td>
<td>464</td>
<td>84</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1998</td>
<td>215</td>
<td>84</td>
<td>3280</td>
<td>546</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1999</td>
<td>273</td>
<td>53</td>
<td>1946</td>
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<tr>
<td>Netherlands</td>
<td>2000</td>
<td>1111</td>
<td>76</td>
<td>5000</td>
<td>354</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2001</td>
<td>863</td>
<td>27</td>
<td>2300</td>
<td>437</td>
</tr>
</tbody>
</table>

Trichothecenes are strong inhibitors of protein synthesis, targeting and damaging, in particular, cells that are actively dividing (thymus, lymph gland, te-
stes, small intestine, spleen). These toxins can, either alone or in combination with each other, cause acute or chronic trichothecene-toxicoses, illnesses characterized by dermatitis, vomiting, food refusal, mucous and skin bleeding, hemorrhagic diarrhoea and sterility. Clinical toxicity picture includes food refusal, vomiting, tachycardia, hemorrhagic diarrhea, edema, skin and mucous necrosis, destruction of hematopoietic tissue, leukocyte and thrombocyte, leukopenia and nervous disorders. Symptoms depend on the type of toxin involved, concentration and exposure time.

Decreased food intake and vomiting are associated with increased activity of central serotonin system in brain. After lower, and especially after higher levels of exposure to deoxynivalenol, reduced growth and development have been reported in most animal species. This has been confirmed by a great number of studies as the most obvious effect of deoxynivalenol toxicity. Two year study conducted on laboratory animals showed that quantity leading to growth and body weight reduction was 0.1 mg/kg of body weight a day. There is no evidence of more significant accumulation of this mycotoxin in the body and body fluid, and detoxification is done through deoxidation, resulting in partial conversion of this mycotoxin in deepoxy DON, and its excretion after conjugation with glucuronic acid.

Deoxynivalenol inhibits DNA and RNA synthesis at a cell level, and protein synthesis at ribosomal level. The toxin has a haemolytic effect on erythrocytes. Higher doses of DON have adverse effect on heart, liver, spleen and thymus.

An issue of great concern is a heat stability of DON, since it is a very stable compound, both during storage and processing, and as such enters the food chain (EFSA, 2004).

GENERAL PRINCIPLES OF HACCP CONCEPT (CAC, 1997)

**Principle 1** Identify the potential hazards

In this stage, HACCP team identifies potential hazards associated with food production at all stages, from primary production, processing, manufacture, and distribution until the point of consumption. After that, the likelihood of occurrence of each hazard needs to be assessed and necessary preventive actions and measures taken to prevent, eliminate or reduce the identified hazard to acceptable levels.

**Principle 2** Determine critical control points (CCP)

Critical control points (CCP) are points, steps, or operational procedures in the cycle of production at which potential hazards may occur, and be eliminated, or reduced to acceptable levels by preventive actions and measures.

Mycotoxins synthesize in the field, and their synthesis continues in bins, and thereafter in storages of finished products. On such a long path, and under various conditions, a number of CCPs can be identified. Some of them are: type and manner of tillage, crop rotation, type of seed (hybrids), weather conditions, presence of moulds and insects, harvest season, drying efficiency, microclimatic conditions in storage facilities.
**Principle 3** Establish the critical limits

A critical limit is a criterion that has to be fulfilled. It separates acceptability from unacceptability. All parameters for different CCPs need to be measured for as short time as possible, to allow timely decisions and corrective actions. The critical limits must be supported by scientifically based data, must be verifiable, measurable and applicable for all CCPs. Sources of information for establishment of critical limits are: laws and regulations, internal requirements, actual practices and experts’ recommendations.

**Principle 4** Establish a monitoring system for each CCP

Monitoring is a scheduled measurement or observation of a CCP relative within its critical limits.

Critical control points are monitored in order to:
- establish when critical limit is exceeded, and human health hazards increased,
- identify the problem before it occurs,
- verify HACCP plan,
- assure and confirm the product quality.

Apart from control, operating limits are also established for the parameters based on which the process is run. They are usually more conservative than control limits, need not be part of HACCP documentation, and are used as a buffer, to adjust the process and prevent critical limit from exceeding.

**Principle 5** Establish corrective actions

Corrective action is any action to be taken when monitoring indicates that critical control points in CCP depart from the critical limits. There are two types of corrective actions: intervention (intermediate) and prevention.

**Principle 6** Establish verification procedures

Verification procedures need to be established to verify whether the HACCP system is working correctly, and whether the identified hazards are within control limits. Verification activities include:
- HACCP system validation
- review of the CCP monitoring results,
- product testing/analysis,
- HACCP system internal audits.

DON level checks must be carried out in regular, prescribed intervals in the field and in the final product to assess whether it is kept within acceptable levels. When there is a deviation from the critical limit, it is considered as a loss of control, and requires adequate actions to be taken. Critical limits may be adjusted, or new control measure must be introduced.

**Principle 7** Establish documentation and record keeping

Efficient and accurate record keeping is essential to the application of an HACCP system, to confirm that the system is working effectively. Records are also used for tracking trends and identifying causes of deviations, for introduction of corrective and preventive actions and measures.
CONCLUSION

Mycotoxins are inevitable food contaminants which enter the food chain at one point or another. In the developed world, advances in food handling and safety procedures have largely eliminated the consumption of food with high levels of mycotoxins, and very few people suffer health problems from dietary exposures to mycotoxins.

Cereals (or wheat) are on the top of the hypothetical warning list regarding the mycotoxin contamination, particularly due to possible health effects on children.

HACCP system is widely recognized as the most effective approach for producing safe food. It is almost a universally accepted method of present and future times. Knowledge and understanding of factors favourable for fungi growth are of crucial importance for successful prevention, since they can reduce exposure of humans and animals to mycotoxins to minimum possible levels. It is still not possible to eliminate completely mycotoxins from food products. Therefore, comprehensive efforts must be made to reduce health risks attributable to mycotoxins to acceptable levels and respond to food safety issues and concerns.

Advantages of HACCP system application are that it reduces foodborne diseases, ensures safety of food, adheres to legislation, inspections and surveillance requirements, enhances competitiveness, removes barriers in international trade, and increases profit.

Implementation of HACCP system is not to be considered as the end of the process. Current maintaining of HACCP plan is to provide real benefits.


LITERATURE


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БЕЗБЕДНОСТИ ПШЕНИЦЕ У ОДНОСУ НА ПРИСУСТВО И САДРЖАЈ ДЕОКСИНИВАЛЕНОЛА

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Резиме

Превенција контаминације хране токсогеним пlesenима је најрационалнија и економски најполоваданија метода за спречавање могућих последица деловања њихових метаболита на здравље људи и животиња. Дексиниваленол (ДОН) припада групи фузарцијумских микотоксинса, оштећује поједине унутрашње органи, док у малим количинама кроз дужи период делује имунотоксиично. Посебно је наглашена изложеност деце овом токсину преко хлеба, а претпоставља се да код њих доводи до смањења телесне тежине и смањене тежине јетре. У овом раду приказан је НАССР концепт у превенцији контаминације пшенице дексиниваленолом. Да би се применио овај систем мора се прво идентификовати опасност и проценити ризик, а за то је неопходна реална слика за подручје (регион) у којем се желе проводити мере превенције. Према резултатима испитивања која се проводе у лабораторији нашег департмана на пшеници са подручја Војводине % контаминираних узорака у 2004. и 2005. години износио је 41,6, а количине дексиниваленола кретале су се од 57 до 1840 μg/kg.