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EFFECTS OF TEMPERATURE AND TIME ON DEOXYNIVALENOL (DON) AND ZEARALENONE (ZON) CONTENT IN CORN

ABSTRACT: Fumonisins are Fusarium mycotoxins that occur in corn and corn-based foods and they have been implicated in several animal and human diseases. Their effect on human health is unclear, however, fumonisins are considered to be risk factors for cancer. Baking, frying, and extrusion cooking of corn at high temperatures (190 °C) reduce fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. The aim of this work was to evaluate the effectiveness of temperature (200 and 220 °C) and time (15 and 20 min) on the detoxification of corn flour deliberately contaminated with DON and ZON. After processing at 200°C for 15 min, an average of 12% and after 20 min an average of 15% of DON was lost. At 200°C ZON content was reduced by 22% (after 15 min) and by 27% (after 20 min). Higher temperature (220 °C) did not significantly affect further reduction of DON or ZON content. The process was only partially effective in both cases.

KEYWORDS: deoxynivalenol, zearalenone, temperature, incubation time.

INTRODUCTION

Fusarium species are widespread in nature, occurring both as facultative saprophytes and parasites of a variety of plants. They are capable of elaborating toxins with varying chemical compositions [Bilgrami and Choudhary 1998]. Various Fusarium species can infect cereal crops under different climatic conditions, the most widely distributed being F. graminearum and F. culmorum.

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Deoxynivalenol (DON) belongs to the trichothecene group of mycotoxins and is formed by fungi of the genus *Fusarium*. DON often occurs in plant products and particularly in cereals. Of the trichothecene mycotoxins, deoxynivalenol, 3-acetyl- and 15-acetyl-deoxynivalenol are the toxins most frequently occurring in Japan, Korea, China, South East Asia, New Zealand and Europe [Ishii 1983; Yoshizawa 1983; International Programme on Chemical Safety 1990; International Agency for Research on Cancer 1993; Yamashita et al. 1995; Lauren et al. 2001]. The toxin concentrations found in wheat, corn or rice are often in the ppm range. Due to their high cytotoxic and immunosupressive properties these toxins pose a risk to human and animal health.

Zearalenone (ZEA) is a non-steroidal oestrogenic mycotoxin that can occur concomitantly with DON since the two compounds are produced by the same *Fusarium* species. ZEA is a phytohormone which displays, apart from its anabolic properties, mainly estrogenic effects. Because of its estrogenic properties, zearalenone may induce fertility disorders in animals with clinical signs of hyperestrogenism – an aspect of disease mainly reported in hogs but also described in other species such as cows, horses and sheep [JECFA 2002; Zöllner et al. 2002].

With the common occurrence of DON and ZEA, it is important to understand not only the factors that lead to their occurrence, but also the management strategies capable of minimizing their impact and occurrence in food products [JECFA 2002]. Numerous guidelines, ranging from altering field conditions to adequate storage practices, have been suggested to prevent mould growth and mycotoxin development in crops [Patey and Gilbert, 1989]. Safe limits of DON in foods ranging from 500 to 2000 ppb have been fixed in Austria, Canada, Japan, Russia, Switzerland and the USA; France and Romania regulation limits for ZEA are 200 and 30 ppb respectively [Food and Agriculture Organization, 1997].

In Serbia safe limits of DON and ZON in foods range from 500 to 1750 ppb and 50 to 350 ppb according to Rulebook on maximum allowed quantities of residue of plant protection means in food and animal food and on food and animal feed for which the maximum allowed quantities of plant protection means are determined [Službeni glasnik RS, 25/2010; 28/2011].

Despite all these efforts, the problem persists. It became necessary to develop processing methods directed at lowering the co-occurring mycotoxins to safe levels and design probabilistic models which consider that processing of raw agricultural commodities may alter the contamination levels in the final products [JECFA 2002].
Table 1. Global occurrence of DON and ZON in 2009, 2010 and 2011. (www.mycotoxins.info)

<table>
<thead>
<tr>
<th></th>
<th>DON</th>
<th>ZON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples tested</td>
<td>2432</td>
<td>2947</td>
</tr>
<tr>
<td>Positive (%)</td>
<td>50</td>
<td>58</td>
</tr>
<tr>
<td>Average (ppb)</td>
<td>831</td>
<td>722</td>
</tr>
</tbody>
</table>

Milling studies evaluating wheat naturally contaminated with *Fusarium* mycotoxins indicate that the mycotoxins were distributed differentially throughout the kernel, with relatively higher levels in the dockage, outer bran fractions, and shorts and lower levels in the inner flour fractions [Scott et al. 1983; Young et al. 1984; Lee et al. 1987]. DON levels were not reduced during the production of Egyptian bread, Western style bread or cookies baked from hard wheat flour [El-Banna et al. 1983; Scott et al. 1983; 1984].

In contrast, the effect of baking on DON in non-yeast products was reported as variable, ranging from no effect to 69% reduction [Young et al. 1984; Abbas et al. 1985]. Additional studies indicated that during bread baking DON was reduced by 49 and 57% [Kamimura et al. 1979]. Chemical treatments have been tested for their effectiveness in reducing DON; in general, better results were obtained at basic pH [Young 1986; Lauren and Smith 2001].

In corn wet milling, ZEA concentrates in gluten (49–56%) and milling solubles (17–26%) but not in starch. All dry-milled corn fractions contained ZEA and only 3–10% were removed by dry cleaning [Scott 1984]. Losses of ZEA added to wheat flour were 34–40% during bread-making, 48–62% when making instant noodles, and 16–27% during processing into biscuits. The decomposition products in bread lacked oestrogenic activity [Matsuura et al. 1981].

The present study was conducted to determine the effects of thermal treatments on DON and ZEA and it wanted to establish the basis for a decontamination model of *Fusarium* mycotoxins in corn.

**MATERIAL AND METHODS**

Ten naturally contaminated corn samples were purchased from Trilogy Analytical Laboratory, 870 Vossbrink Dr. Washington, MO63090. Samples were stored at 4 °C until used. The presence of ZON and DON was studied by *enzyme-linked immunosorbent assay* (ELISA) method. To obtain a whole corn powder, samples were ground to pass through 0.5 mm mesh; the remaining
fraction was ground consecutively since no more than 4% was bigger than 0.5 mm. All fractions were pooled and thoroughly mixed.

**Heating treatment:** All samples were heated in an electric convection oven at 200 and 220 °C for 15 and 20 min (a total of 24 treatments). Samples (10 g) were put in aluminium vessels (5.5 cm i.d. x 3.5 cm height), extended to form a 1 cm high layer and placed into the oven preheated to the selected temperature. After the treatment, the samples were immediately cooled and weighed.

**Chemicals and mycotoxins standards:** All reagents were purchased from R-biopharm. Test kit for DON determination contains:
- 1 microtiter plate with wells coated with capture antibodies against anti-DON antibodies,
- 1 DON standard solution, 1.3 ml 0 ppm (zero standard) in water, 1 peroxidase-conjugated DON, 1 anti-DON antibody, 1 substrate/chromogen, 1 stop solution containing 1 N sulfuric acid and 1 Washing buffer (Salt) for preparation of a 10 mM Phosphate Buffer (pH 7.4) containing 0.05% Tween 20.

Test kit for ZON determination contains:
- 1 microtiter plate with wells coated with capture antibodies against anti-zearalenone antibodies, 1 Zearalenone standard, 1.3 ml 0 ppb (zero standard) in methanol/water, 1 peroxidase-conjugated zearalenone, 1 anti-zearalenone antibody monoclonal, 1 Substrate/chromogen and 1 Stop reagent containing 1 N sulfuric acid.

**Sample preparation:**
DON analysis: weigh 5 g of ground sample, put it into a suitable container and add 100 ml of distilled water; blend the sample by ultra-turrax (or equivalent) for two minutes or shake vigorously for three minutes (manually or with shaker); filter the extract through Whatman No. 1 filter (or equivalent); use 50 μl of the filtrate per well in the test.
ZON analysis: weigh 5 g of ground sample and add it to a suitable container with 25 ml of methanol (70%); shake vigorously for 3 min (manually or with shaker); filter the extract through Whatman No. 1 filter (or equivalent); dilute 1 ml of the obtained filtrate with 1 ml of distilled or deionized water; use 50 μl of the filtrate per well in the test.

**Test procedure:**
DON analysis:
1. Insert a sufficient number of wells into the microwell holder for the standard and samples to be run. Record standard and sample positions.
2. Pipet 50 μl of standard or prepared sample into separate wells; use a new pipette tip for the standard or each sample.
3. Add 50 μl of enzyme conjugate to the bottom of each well.
4. Add 50 μl of anti-DON antibody solution to each well. Mix gently by shaking the plate manually and incubate for 5 min (+/-1) at room temperature (20–25 °C / 68–77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette fill the wells with distilled or deionized water (250 μl per well). Empty the wells again and remove all remaining liquid. Repeat the washing steps two more times.
6. Add 100 μl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 3 min (+/-0.5) at room temperature (20–25 °C / 68–77 °F) in the dark.
7. Add 100 μl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes after the addition of stop solution.

ZON analysis:
1. Insert a sufficient number of wells into the microwell holder for the standard and samples to be run. Record standard and sample positions.
2. Pipet 50 μl of standard or prepared sample into separate wells; use a new pipette tip for the standard or each sample.
3. Add 50 μl of enzyme conjugate solution to each well.
4. Add 50 μl of anti-zearalenone antibody solution to each well. Mix gently by shaking the plate manually and incubate for 10 min (+/-1) at room temperature (20–25 °C / 68–77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette fill the wells with distilled or deionized water (250 μl per well). Empty the wells again and remove all remaining liquid. Repeat the washing steps two more times.
6. Add 100 μl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 5 min (+/-0.5) at room temperature (20–25 °C / 68–77 °F) in the dark.
7. Add 100 μl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes.
RESULTS AND DISCUSSION

All corn samples examined were contaminated with deoxynivalenol (DON) in concentration of 1.9 ppm and with ZON in concentration of 91.1 ppb.

After heating treatment at 200 ºC for 15 and 20 minutes the results showed the reduction of DON concentration by 11.58% and 15.26% respectively (Figure 1), and the reduction of ZON concentration by 21.79% and 26.65% respectively (Figure 2).

Figure 1. DON concentration in naturally contaminated corn flower after heating treatments

By increasing the temperature of the heating treatment to 220 ºC the reduction of the DON and ZON concentration after 15 and 20 minutes was not significantly different from the level of DON concentration from the previous case (Figure 1). However, the level of ZON decreased up to 21.44% and 27.68% respectively (Figure 2).

Figure 2. ZON concentration in naturally contaminated corn flower after heating treatments
Similar examinations conducted on samples of grain powder by Yumbe-Guevara et al. (2003) showed the reduction of DON concentration after heating treatments for 60 minutes at temperatures of 140 °C, 160 °C, 180 °C, 200 °C and 220 °C were by 7.5%, 24%, 38%, 48% and 50% respectively. Not significantly different results regarding ZON concentration reduction were obtained.

A large number of other examinations that included heating treatments while baking samples were conducted and showed various results. Young et al. [1984] treated samples of soft white winter wheat, naturally contaminated with DON (range: 0.28–0.44 ppm), using a baking cookies standard receipt that included heating treatment and the results showed that DON level was decreased up to 35%. On the other hand, Lancova et al. [2008] showed that baking at 210 °C for 14 minutes had no significant effect on DON levels in examined samples (DON range 0.09–2.99 ppm). Different and sometimes contradictory results were presented by Samarajeewa [1991] in her studies which stated that low penetration of heat, which varies from sample to sample, may be the reason for various results.

CONCLUSION

Reduction of DON and ZON concentrations in examined corn powder samples simple by their exposure to high temperature can be achieved to the level of 15% and 27% respectively. This level of reduction for itself cannot be sufficient. However, it can be very significant and taken into consideration if heating treatment is used as a part of food processing in which the concentration of DON and ZON in the processes of cleaning and wet and dry milling are already reduced to some level.

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Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja (2010. i 2011). Službeni glasnik Republike Srbije, 25/2010 i 28/2011. (Sr)


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**УТИЦАЈ ТЕМПЕРАТУРЕ И ВРЕМЕНА НА САДРЖАЈ ДЕОКСИНИВАЛЕНОЛА (ДОН) И ЗЕАРАЛЕНОНА (ЗОН) У КУКУРУЗУ**

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**РЕЗИМЕ: Фумонизини су микотоксини рода Fusarium, који се јављају код кукуруза и прехрамбених производа на бази кукуруза и укључени су у неколико животињских и људских обољења. Иако је њихов утицај на људско здравље нејасан сматрају се фактором ризика код појаве рака. Печење, пржење и екструзија кукуруза на вишим температурама (190 °C) такође смањује концентрацију фумонизина у храни, а количина оствареног смањења зависи од времена кувања, температуре, рецептуре и осталих фактора. Циљ овог рада је био да процени ефективност температуре (200 и 220 °C) и времена (15 и 20 мин.) на детоксикацију кукурузног брашна које је било контаминирано са деоксиниваленолом и зеараленоном. Просечно смањење концентрације деоксиниваленола након третмана на 200 °C у трајању од 15 мину-
та износило је 12%, а након трајања од 20 минута 15%. Просечно смањење концентрације зеараленона након третмана на 200 °C у трајању од 15 минута износило је 22%, а након трајања од 20 минута 27%. Више температуре (220 °C) нису значајно утицале на даље смањење концентрација деоксиниваленола и зеараленона. Третман је само парцијално утицао у оба случаја.

КЉУЧНЕ РЕЧИ: деоксиниваленол, зеараленон, температура, период инкубације