ABSTRACT: Mycotoxins are highly toxic compounds produced by molds that commonly occur in cereal grains. These toxins can have adverse effects on human and animal health. Mycotoxin decontamination procedures can be divided into three different groups: chemical, biological and physical. Procedures from the first two groups are often regarded as unacceptable for lowering the mycotoxin level. A laboratory brush was developed for the purpose of physical cleaning of corn kernel. Three samples of commercially available corn kernels were subjected to the brushing procedure in order for the experimental study to be conducted. The mass of 100g of corn kernels was placed on motionless screen of the brush. A rotating part of the experimental device, the polypropylene bristle brush was set to higher speed (higher than 800 rpm). During the corn brushing, dust and broken kernels were brushed out through the motionless screen. Corn samples were taken before and after the brushing procedure and they were analyzed for aflatoxin concentration by HPLC-UV RED. By comparing the control and brushed samples, it can be noticed that removal of fines caused the reduction in the level of mycotoxins in all three brushed samples.

KEY WORDS: mycotoxins, corn, brushing

INTRODUCTION

Mycotoxins are highly toxic compounds produced by molds that commonly occur in cereal grains. They are produced by molds (fungi) under field conditions and harvest, or during storage (M a r g u a r d t, 1996; S t o j a n o v i ć et al., 2005). Mycotoxins can have adverse effects on human and animal health by causing toxic response when ingested. Intake of contaminated material potentially induces acute and chronic diseases, which may result in teratogenic, carcinogenic, and immune-suppressive effects (W u , 2004; P e s t k a , 2007, J a j i ć et. al., 2008).
Mycotoxins are thermostable molecules which can be totally destroyed only at very high temperatures, which have deteriorating effects on nutritive value of contaminated material. Therefore, value parameters (such as temperature and pressure) in standard procedures applied to food and feed processing are not normally as high as it is required for total decontamination of material. However, many of those processes affect the reduction in mycotoxins (Scott, 1984; Erdington et al., 1997, Buleman and Bianchini, 2007).

With respect to the applied processes, mycotoxin decontamination procedures can be divided into three main groups: chemical, biological, and physical. Chemical substances used for decontamination of material are acids, alkali, oxidizing reagents, reducing agents, and chlorinating agents. These substances can cause mycotoxin content to be reduced by up to 99%. Although they are very effective, chemical treatments are not widely used due to practical problems: they are expensive and time consuming; they can change palatability and nutritive value of material; they decrease material quality, and can induce the formation of toxic by-products (Hameed, 1993, Avantaggio, 2012).

Biological detoxification is also used for decreasing of mycotoxin content in material. The main action of these agents is biotransformation and/or biodegradation of mycotoxins by microorganisms or enzymes, which results in production of non-toxic metabolites. After mixing with contaminated material, these bio-adsorbents must be activated in gastrointestinal tract of the animal. Therefore, the effects of their addition could not be noticed before the intake of material (Dänicke, 2004, Avantaggio, 2012).

Physical procedures include screening, sorting, removal of infested grains, γ irradiation, thermal treatments, adsorbing, etc. Most of the food and feed production processes can be considered as physical. By milling and fractionation of milled material, mycotoxin contamination may be redistributed and concentrated in certain milling fractions. However, this procedure could not totally remove mycotoxins (Abbas et al., 1985, Scudamore et al., 2003). Thermal processing can also be used for decontamination. However, there is a difference in effects on mycotoxin content depending on the specific process. For example, cooking, baking or roasting have inappreciable effects on toxin content. On the other hand, extrusion cooking process, apart from high temperature, includes high pressures and shear forces. Under these conditions, mycotoxin content can be reduced. Although extrusion process can be effective, there are high investment and operational costs for the implementation of this process (Ryu et al., 2003; Scudamore et al., 2004).

The aim of this experimental work was to investigate the influence that removal of fines by brushing process can have on the reduction of aflatoxin level in corn.
MATERIAL AND METHOD

Three samples of commercially available corn kernels were subjected to a brushing procedure for the purposes of this experimental study. A laboratory brush was developed for the purpose of physical cleaning of the kernels. The mass of approximately 100g of corn kernels was placed on motionless screen of the brush. A rotating part of the experimental device, the polypropylene bristle brush was set to higher speed (higher than 800 rpm). During the corn brushing, dust and broken kernels were brushed out through the motionless screen.

Corn samples were taken before and after the brushing procedure and they were analyzed for aflatoxin level by HPLC-UV RED. The basic solution containing AFLATOXINS LC Tech P/N 10837 (aflatoxin B1 2.02 µg/ml and aflatoxin B2 0.5 µg/ml) of acetonitrile was used for the determination of aflatoxin content. The basic standard (1 ml) was diluted to 50 ml with mobile phase. Samples were homogenized and part of it was extracted with methanol/water mixture. Afterwards, the extract was filtrated and an aliquot was diluted in PBS. Immuno-affinity column (AlfaCLEAN, 3ml widebore) was used for the purification of prepared samples. Column was washed out with distilled water and air stream dried. Elution was done in methanol; eluate was dried in the nitrogen stream and reconstructed with mobile phase. The prepared samples were analyzed with HPLC/FLD. The HPLC analyses were carried out on 1100 Agilent system with Zorbax EclipsePlus C18 (150x4.6 mm, 3.5 µm) column. The mobile phase used was: (A) water and (B) acetonitrile in gradient mode, with the flow rate of 1.54 ml/min. The injection volume was 50 µl. The detection limit for aflatoxin B2 was 0.05 ng/ml, while for aflatoxin B1 it was 0.2 ng/ml. The quantification limit for aflatoxin B2 was 0.1 ng/ml, while for aflatoxin B1 it was 0.4 ng/ml. The average recovery rate (n=3) for aflatoxin B1 was 86.3% and for aflatoxin B2 it was 83.4%. The repeatability was in accordance with the European Commission (EC) regulation No. 401/2006, with the RSD less than 30%. The triness for aflatoxin B1 was -19% and for aflatoxin B2 it was -8%, which was also in accordance with the EC decision 2002/657/EC.

STATISTICA software version 9 (Statsoft, Tulsa, OK, USA) was used for Tukey’s HSD comparison of means of samples. Single-factor ANOVA calculations were used for comparing samples before and after the brushing procedure. Differences among means with probability value of p ≤ 0.05 were accepted as statistically significant differences, and differences among means with the value of 0.05 ≤ p ≤ 0.10 were accepted as tendencies to differences.

RESULTS AND DISCUSSION

Figure 1 shows the concentration of aflatoxin B1 in unbrushed and brushed samples. Logarithmic distribution is used for expressing the values of aflatoxin B1 concentration (µg/kg). Concentration of aflatoxin B1 in all samples was very high before physical cleaning process, and it ranged from 199.7
70 to 263.5 μg/kg. The application of brushing process had significant influence (p < 0.05) on reducing the content of aflatoxin B1 in all three samples. The use of brushes for corn kernel surface cleaning induced the reduction of the specified mycotoxin content from 57.0 to 98.7% (Fig. 2).

Fig. 1 – Concentration of aflatoxin B1 in unbrushed and brushed samples. The values are presented as mean values, n = 3. All treated samples have significantly (p ≤ 0.05) lower aflatoxin content.

Fig. 2 – Percentage of removal of aflatoxin B1 in brushing process.
Figure 3 shows the concentration of aflatoxin B2 in unbrushed and brushed samples. It can be seen that for the sample 1 concentration of aflatoxin B2 content was reduced from 7.0 to 4.4 μg/kg. By expressing the results in percentages, aflatoxin B2 was removed from the sample 1 by 36 % (Fig. 2). Aflatoxin B2 content in samples 2 and 3 was reduced from 19.4 μg/kg (sample 2) and 7.8 μg/kg (sample 3) to 0.1 and 0.2 μg/kg, respectively, which was lower by 99.6 (sample 2) and 97.4% (sample 3).

P a r k and L i a n g, 1993, reported the results obtained from the removal of aflatoxin from peanut by applying physical procedures, such as cleaning and segregation. They applied six different procedures for decontamination. Cumulative reduction, achieved by all procedures, was 99.3 %. Removal of aflatoxin per procedure is presented in Table 1.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Aflatoxin concentration (μg/kg)</th>
<th>Reduction (%)</th>
<th>Cumulative reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmers’ stock</td>
<td>217</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Belt separator</td>
<td>140</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Shelling plans</td>
<td>100</td>
<td>29</td>
<td>54</td>
</tr>
<tr>
<td>Color sorting</td>
<td>30</td>
<td>70</td>
<td>86</td>
</tr>
<tr>
<td>Gravity table</td>
<td>25</td>
<td>16</td>
<td>88</td>
</tr>
<tr>
<td>Blanching/color sorting</td>
<td>2.2</td>
<td>91</td>
<td>99.0</td>
</tr>
<tr>
<td>Re-color sorting</td>
<td>1.6</td>
<td>27</td>
<td>99.3</td>
</tr>
</tbody>
</table>

Fig. 3 – Concentration of aflatoxin B2 in unbrushed and brushed samples. The values are presented as mean, n = 3. All treated samples have significantly (p≤0.05) lower aflatoxin content.
Although high reduction results were obtained, several unit operations were combined in this process and they required high investment costs for equipment purchasing.

Park, 2002, used physical cleaning for the reduction of aflatoxin content in corn. By removing kernels with extensive mold growth and cleaning of corn kernels, 40% to 80% of reduction was achieved. This author also used dry milling process for fractionation of aflatoxin B1 content. The highest levels of mycotoxin were found in germ and hull fractions. Grits, low-fat meal and low fat flour contained only 6-10% of aflatoxin. However, this type of operation can be used only for the concentration of toxin in separate fractions, and not for removal from overall mass.

Conway et al., 1978, reduced aflatoxin content in corn by microwave roasting. In comparison with physical procedures, microwave roasting requires high energy consumption.

Aваттіято, 2012, reported that screening/cleaning process could achieve significant reduction of aflatoxin content (by 30-40%). The same author reported that corn dehulling could cause the reduction of aflatoxin content by up to 93%, and with density segregation in floating water and/or saturated NaCl solution, aflatoxin could be reduced by up to 70%.

When comparing the experimental results with the data from literature, it can be noticed that brushing process used in this study appeared to be very effective in aflatoxin reduction. When it is applied to remove mycotoxin, this procedure does not alter physical or chemical status of the kernel, as it is the case with most procedures applied to lowering of mycotoxins. Additionally, comparing with other processes used for decontamination of material, investment and operational costs for utilization of brushing process are considerably low.

Fig. 4 – Percentage of removal of aflatoxin B1 in brushing process.
CONCLUSION

Results obtained from this study showed that application of brushing process to mycotoxin removal from corn kernel caused significant ($p < 0.05$) reduction of aflatoxin content. The brushing process reduced aflatoxin B1 content in the range from 57.0 to 98.7%, while aflatoxin B2 was reduced from 36% to 99.6%, depending on the sample. This is above the values reported in the literature for most of the other decontamination procedures. By applying brushing process, physical and chemical characteristics of corn kernel remained unchanged, in contrast to many other processes used for mycotoxin removal.

REFERENCES


СНИЖАВАЊЕ НИВОА МИКОТОКСИНА У КУКУРУЗУ УКЛАЊАЊЕМ ЧЕСТИЦА ПРАШИНЕ

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Резиме

Микотоксини су веома токсична јединиња која продукују плесни, а најчешће се појављују у житарицама. Ови токсини могу негативно утицати на људско и животињско здравље. Поступци за деконтаминацију материјала заражених микотоксинима могу се поделити у три групе: хемијски, биолошки и физички. Хемијски и биолошки поступци често нису прихватљиви за снижавање нивоа микотоксина у сировинама и храни. За потребе физичког чишћења кукурузног зрна развијена је лабораторијска четкалица. У експерименталном раду узета су три комерцијално доступна узорка кукуруза у зрну, која су подвргнута поступку четкања. Маса од око 100 грама зрна кукуруза је стављена на непокретну перфорирану површину. Ротирајући део експерименталног уређаја, полипропиленска четка је постањала на велики број обрта (већи од 800 обрта у минути). Током четкања зрна кукуруза прашина и сломљена зрна су одстрањена кроз отворе на перфорираној површини. Пре и после четкања узети су узорци зрна кукуруза, који су анализирани на садржај афлатоксина поступком високопритисне течне хроматографије (HPLC-UV RED). Упоређивањем контролних и четканих узорака може се претићи да је уклањање прашине изазвало снижавање микотоксина код сва три трећираних узорака.

КЉУЧНЕ РЕЧИ: микотоксини, кукуруз, чишћење
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