Microbial and mycotoxin contamination of maize during storage

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Abstract

Production and trading of good quality cereals is very important, because microbiological and mycotoxin contamination represents a risk for human and animal health. The best way to protect consumers against toxic effects of fungi and mycotoxins from cereals is to apply the HACCP system for production and storage.

As maize quality before storage and storage conditions are of paramount importance, we assessed microbial and mycotoxin contamination of 11 maize samples, comprising of different combinations of heathy and damaged corncobs. Tested parameters were bacterial and fungal and mycotoxin levels (deoxynivalenol, zearalenone and total aflatoxins), taking into account relative humidity (% RH) and temperature, for the 7 months storage period of maize samples. Results show that microbial and mycotoxin contamination of maize is dependent on temperature and RH conditions.

Keywords: maize, storage, contamination, microbiology, mycotoxins

Introduction

F. graminearum is a fungus which is contaminates cereals in field and produces *Fusarium* head blight (FHB) in wheat and Ear rot in maize. Fusarioses lead to a decrease of productivity and alter grain quality during milling and processing. The mycotoxins deoxynivalenol (DON, vomitoxin) and zearalenone (ZON), which are produced by *Fusarium*, are stable during cereal storage, milling and processing. Mainly, DON can produce gastrointestinal and hematological problems in most animals, being a potential inhibitor of proteic synthesis in eukariotic cells (9). ZON has carcinogenic, estrogenic and anabolic properties; it can produce fertility and reproducibility disorders especially in pigs (4). This mycotoxin is not classified as carcinogenic in humans, being included in group 3 by IARC (International Agency for Research on Cancer), still has been associated with precocious sexual development of children and breast enlargement in boys (1). Biological effects of ZON impose its routine monitoring in feed and food (8).

Aflatoxins [**A** (*Aspergillus)* + **FLA** (*flavus*) + **toxin**] are storage mycotoxins, mostly problematic in maize production. In animals and humans, aflatoxins have chronic and acute toxic effects, affecting liver (causing necrosis, cirrhosis and carcinomas); acute symptoms include queasiness, abdominal pain, and lung edema.

Seasonal pattern of fungal toxicosis in livestocks reveal *Fusarium, Aspergillus, Penicillium* as high levels of feed contamination with *Fusarium* during autumn as these fungi belong to field and intermediate microflora. The highest incidence of *Aspergillus* occurs during spring and decrease in other seasons.

Fungi have the ability to grow in extremely dry conditions, if additional obstacles do not occur (cooling, chemical agents with antifungal activity, modified atmosphere).

Surprisingly, few studies were performed on interaction between species epiphytic microbial community of stored cereals. Because of number oscillations, quantitative evaluation of cereal microbiota is very difficult; in good quality cereals the number of spored bacteria is not higher than 10^3 cfu/g and represents around 1 % of total number of bacteria. In case of spoiled cereals, because of heating process, the number of spored bacteria can reach 10^5 cfu/g. In good quality cereals the number of moulds can be between 0.5 – 3 x 10^3 cfu/g; a higher value denotes an alteration risk (3).

Development of toxigenic fungi on stored cereals during 5 months depends on anatomical structure of grains, integrity and quality of microbiota, moisture (over 20 %), relative humidity of air (60 % RH), temperature (25 – 45^0C), fungi, ratio of broken kernels, insects and oxygen (1, 13, 6).

A study showed that all fungal species were able to grow in atmosphere with 80 % CO_2 and 20% O_2, but growing was lower than in air, especially for *Penicillium* spp. and *Aspergillus flavus*; atmosphere composition had a marked effect on mycotoxin production than growing (5, 6).

Mycotoxins production in *Fusarium* contaminated wheat during an eight month storage period was studied by Homdork (7); results showed that DON level remained constant and zearalenone increased to the end of the storage period at 25^0C and 90 % RH.

To prevent and reduce contamination of cereal with fungi and mycotoxins during storage, the following conditions must be respected (2): forced aeration of storage places, dry storage places, prevention of rodents and birds, detection of mycotoxin levels, checking of humidity and temperature in storage places (at 1 – 2 weeks), using of approved insecticides and fungicides; fumigation has not effect on infected grains (14); using of preservatives (organic acids: propionic acid etc.) which have antifungal effect and prevent mycotoxin production in cereals used for foods; using well-informed procedures for harvest and storage, which must be implemented each season. These information are helpful to explain the reasons of fungal growth and mycotoxin production in a certain year, and also to avoid similar errors in the future.

Important economic losses were due to rejection of cereals exceeding maximum contamination limits. Discarding of contaminated crops also arise financial issue.

Mixing of healthy and contaminated grains is not compatible with legal stipulations to decrease mycotoxins in food. Moldy smell is kept in flour and food, which become improper to eat. Furthermore, use of contaminated cereals for feed can favor mycotoxins transfer in meat, milk or eggs.

Aim of this study was to determine the effect of storage conditions on the microbial and mycotoxin contamination of maize processed for feed and food.

Materials and Methods

For experiments, 11 maize samples were obtained by mixing healthy and damaged maize [obtained by deep plough-land and presented the highest microbial contamination (bacteria: 2.3 x 10^5 cfu/g; moulds: 1.5 x 10^5 cfu/g)] (table 1). These 11 maize samples were stored at SCDA Podul Iloaiei and analyzed for bacteria, moulds and mycotoxins contamination during 7 months.

Table 1. Maize samples (% healthy cobs + % damaged cobs) stored during 7 month period

Maize sample	Healthy cobs, %	Damaged cobs, %
V1	100	0
V2	90	10
V3	80	20
V4	70	30
V5	60	40
V6	50	50
V7	40	60
V8	30	70
V9	20	80
V10	10	90
V11	0	100

Detection of microbiological contamination was performed accordingly SR ISO 7698:1994. Cereals, pulses and derived products: Enumeration of bacteria, yeasts and moulds.

Corn cob mixtures were ground using a Retsch ZM 200 ultracentrifugal mill (18000 rpm, fitted with a conidur sieve of 2 mm) and obtaining full fat maize flour as processed product.

Mycotoxin detection and quantification from maize flour was performed by ELISA method, using RIDASCREEN®DON test kit (ppb = µg/kg), RIDASCREEN®Zearalenon test kit (ppt = ng/kg) and RIDASCREEN®FAST AFLATOXIN SC test kit, according to the R - Biopharm procedures (10, 11, 12). Absorbance was measured at 450 nm, by a Sunrise microplate reader (Tecan, Austria), fitted out with RIDAWIN software (R - Biopharm).

Results

Results showed that sample V1 (100 % healthy corncobs) had the best evolution because bacterial population decreased from 10^5 to 10^3 cfu/g, while increasing of bacterial contamination starting from V6 (50 healthy cobs + 50 % damaged cobs) (fig. 1). For V9 (20 % healthy cobs + 80 % damaged cobs), bacterial contamination was very high. The most evident increase of bacterial contamination was registered after a 5 months storage period, subsequent to which bacterial population decreased.

Regarding evolution of bacterial contamination of the 11 maize samples, the highest contamination level was registered for V6 (50 % healthy cobs + 50 % damaged cobs); for sample V6, bacterial contamination increased 33.9 times between 3rd and the 5th month, after that it stagnated. Also, for sample V7 (40 % healthy cobs + 60 % damaged cobs), bacterial contamination increased 33 times between 5th and the 7th month.

Mould contamination was found higher beginning with sample V5 (60 % healthy cobs + 40 % damaged cobs). Sample V6 (50 % healthy cobs + 50 % damaged cobs) had the highest level of contamination after 5 months of storage (October – February), after that it decreased. Regarding mould evolution in maize samples, the highest level of contamination was registered for V8 (30 % healthy cobs + 70 % damaged cobs); mould contamination increased 3.6 times between 3rd and 5th month of storage and 13.5 times between 5th and 7th month. Related to the period 3rd – 7th month (January – April), mould contamination increased 49 times. Even if sample V11 was represented by 100 % damaged cobs, it had a lower evolution in mould contamination [increasing 1.4 times in 3 – 5 month, 5.2 times in 5 – 7 month and 7.6 times in 3 – 7 month (January – April)].

Deoxynivalenol and total aflatoxins were not detected in maize samples.

It was very difficult to interpret the results of zearalenone detection from maize samples stored 7 months. Because of this situation, an average concentration of zearalenone was calculated to determine the influence of mixing proportion for healthy and damaged maize cobs (fig. 3).

It was seen that zearalenone concentration increased with percentage of damaged cobs. The highest concentration of zearalenone was detected in V6 (50 % healthy cobs + 50 % damaged cobs) for 4 – 7 months of storage period (68 µg/kg) and V11 (100 % damaged cobs) (63 µg/kg). ZON was detected in all samples; there was a correlation between bacterial and fungal contamination, especially for V5, V6, V7, V10 and V11. To have a graphic representation, ZON concentrations (ng/kg) were transformed in µg/kg.

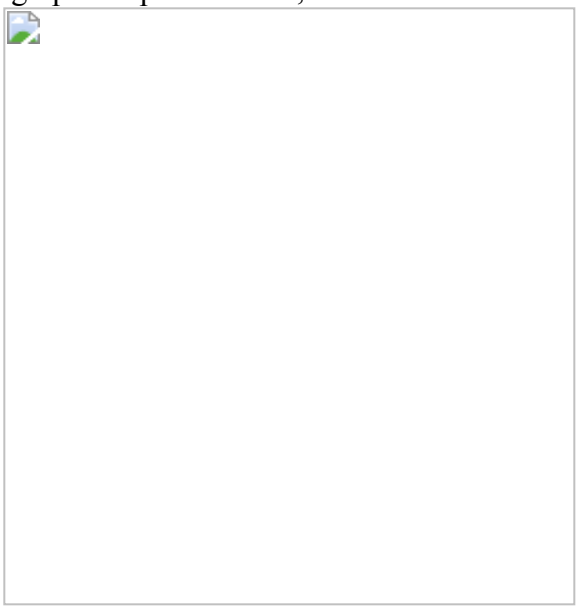

Figure 1. Evolution of bacteria in maize sample during 7 months of storage

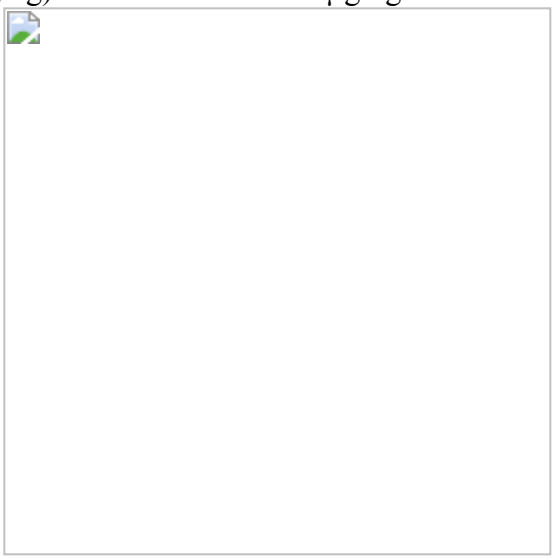

Figure 2. Evolution of moulds in maize sample during 7 months of storage

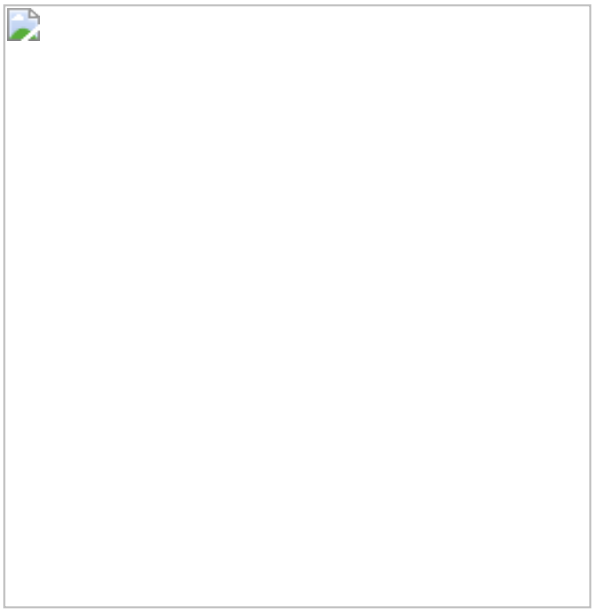

Figure 3. ZON contamination (mean concentration) of maize samples, during 7 month of storage

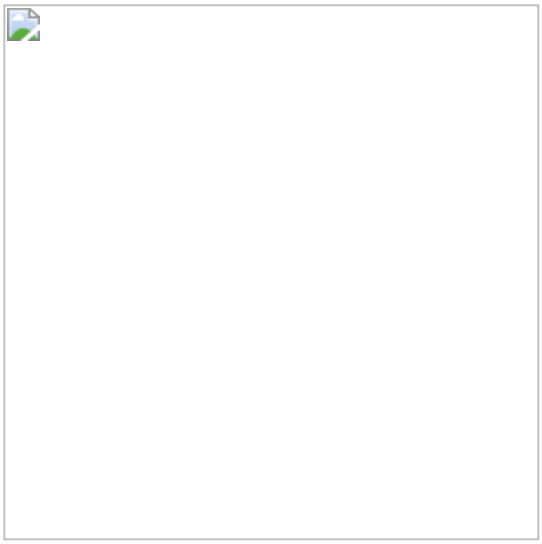

Figure 4. Evolution of relative humidity (% RH) and temperature during storage of maize samples

There was a close correlation between bacterial and mould contamination of maize samples and relative humidity (% RH) in the storage place (fig. 1, 2, 4). The highest RH values were recorded between the 4^{th} and the 5^{th} month with an average value of 81 %, which was 28.4 % higher than for the first 3 months and 13.6 % higher than for 7 months of storage; in the first 5 months, these RH values led to an increase of bacteria and mould numbers and their metabolic activity (CO_2 and ZON production).

Discussions

Analysis of maize contamination with bacteria and moulds showed that 10 – 40 % damaged cobs may be used for mixing with healthy cobs. Maize samples with 50 – 100 % damaged cobs presented a high contamination with bacteria and moulds after 5 – 7 months of storage period.

These maize samples were not contaminated with deoxynivalenol and total aflatoxins. For samples with 50 % to 100 % damaged cobs, ZON concentrations (mean values on 7 months of storage period) increased with bacterial and fungal contamination. Nevertheless, detected ZON concentrations are lower than maximum limits established in Official Gazette no. 1056 bis/2005 (ZON - 200 µg/kg; DON - 1.750 µg/kg; total aflatoxins - 4 µg/kg).

Microbiological and mycotoxin contamination of maize samples was correlated with temperature and relative humidity of air from the storage place; this corelation proved that it is very important to record these parameters.

Conclusions

Obtained results showed that the most efficient combinations of healthy and damaged maize cobs were the following: (60 ... 90 %) healthy cobs + (40 ... 10 %) damaged cobs. In case of a temperature and RH increase in storage places, microbial and mycotoxins contamination will render them improper.

Acknowledgments

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