RESEARCH REGARDING THE INFLUENCE OF LOW TEMPERATURE CEREAL STORAGE ON THE PRODUCTION OF AFLATOXINS

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Abstract
At the current moment cereals represent the main food source as well for humans as for animals. In the previous years, the cereal global production has been facing disastrous effects induced by many toxicogenic species. Their impact manifests in favourable attack conditions, especially through massive reduction of the harvest. According to the FAO (Food and Agriculture Organisation of the United Nations) there is an annual loss of more than 20% of the world's cereal harvest. The main part of this loss is due to insect activity and mould growth. The current practice has proven that the influence of secondary metabolites (mycotoxins), which come as a result of the infection, upon the seed quality surpasses in gravity the quantitative order economical losses. The cereal diseases produced by moulds have the greatest implications towards reducing the harvest, reducing the technological properties of the grains and also due to their harmful activity for man and animal. The harmfulness of grains and of products derived from their processing is mainly determined by the presence of mycotoxins produced by moulds, which attack the plants during vegetation, harvesting or storage and are transmitted to the grains and to the processed products designed for consumption.
Mycotoxins produced by different species of Aspergillus (A. flavus, A. parasiticus, A. versicolor, A. niger etc.)are known under the name of aflatoxins and are representative of secondary metabolites elaborated after the logarithmic multiplication faze of micets. They can be produced by some species of Penicillum and Rhizopus.
One of the factors which has an influence on the activity of toxicogenic moulds is temperature. The current study analyzes cereals from the perspective of storage temperature influence on mycotoxins production.
The laboratory analyzes done for the qualitative and quantitative determination of mycotoxins from the samples, were carried out at the Laboratory for residues belonging to Sanitary Veterinary and Food Safety Department Galati using the Enzyme-Linked Immunosorbent Assay (ELISA) method with the Aflatoxin RIDASCREEN SET kit.
The purpose of the research is to determine the cereal optimum storage temperature so as to obtain a reduction of aflatoxin development.

Keywords:cereals, aflatoxins, temperature, storage

1. INTRODUCTION

At the current moment cereals represent the main food source as well for humans as for animals. In the previous years, the cereal global production has been facing disastrous effects induced by many toxicogenic species. Their impact manifests in favourable attack conditions, especially through massive reduction of the harvest [7]. According to the FAO (Food and Agriculture Organisation of the United Nations) there is an annual loss of more than 20% of the world's cereal harvest. The main part of this loss is due to insect activity and mould growth [4]. The current practice has proven that the influence of secondary metabolites (mycotoxins), which come as a result of the infection, upon the seed quality surpasses in gravity the quantitative order economical losses [5]. The cereal diseases produced by moulds have the greatest implications towards reducing the harvest, reducing the technological properties of the grains and also due to their harmful activity for man and animal. The harmfulness of grains and of products derived from their processing is mainly determined by the presence of mycotoxins produced by moulds, which attack the plants during vegetation, harvesting or storage and are transmitted to the grains and to the processed products designed for consumption [2].

Mycotoxins produced by different species of Aspergillus (A. flavus, A. parasiticus, A. versicolor, A. niger etc.)are known under the name of aflatoxins and are representative of secondary metabolites elaborated after the logarithmic multiplication faze of micets. They can be produced by some species of Penicillum and Rhizopus [3,6].
One of the factors which has an influence on the activity of toxicogenic moulds is temperature [1]. The current study analyzes cereals from the perspective of storage temperature influence on mycotoxins production.
The purpose of the research is to determine the cereal optimum storage temperature so as to obtain a reduction of aflatoxin development.

2. MATERIALS AND METHODS

In the framework of the experiments we have used wheat with 14% moisture content from companies in Galati area. The wheat sampling was done according to the provisions of the EC Regulations nr. 401/2006, and provisions of EC Regulations nr. 1881/2006 [9].
The laboratory analyzes done for the qualitative and quantitative determination of mycotoxins from the samples, were carried out at the Laboratory for residues belonging to Sanitary Veterinary and Food Safety Department Galati using the Enzyme-Linked Immunosorbent Assay (ELISA) method with the Aflatoxin total RIDASCREEN SET kit and RIDASCREEN Aflatoxin B_1 30/15 SET kit.
The principle of the test is the antigen-antibody reaction. The orifices from the wells of the microplate are covered with captivation antibodies directed towards the anti – aflatoxin antibodies. The standard or the sample solution, the conjugated aflatoxin –enzyme and the anti – aflatoxin antibody are added. The free and conjugated enzymes obligatorily compete for the aflatoxin antibodies on sites (competitive immunologic test). At the same time the aflatoxin antibody is also bound to the immobilized captured antibodies. Some unbound aonjugated enzymes are then passed again through the washing step. The enzyme substrate (urea peroxide) and the chromogenic one (tetramethylbenzidine) are added over the wells and incubated. The bound conjugated enzymes transform the colour of the chromagen into a blue product. The adding of the stopping solution leads to the change of colour from blue to yellow. The measurement is done at 450 nm; the absorption is reversely proportional to the sample aflatoxin concentration [8].

Necessary materials and apparatus

Apparatus
- ELISA spectrophotometer (450 nm)
- ELISA spectrophotometer microplate
- laboratory ratchet
- stirrer
- filter paper and funnel
- various graded pipettes: 20µl – 200µl and 200µl – 1000µl

For the evaluation of the results given by the RIDASCREEN enzymatic test, RIDASOFT special software is used.

3. RESULTS AND DISCUSSION

We have monitored the total aflatoxin and B_1 aflatoxin concentrations from horizontal storehouses depending on the temperature variation.
The determination of the total aflatoxin content from the wheat samples taken from the horizontal storehouses led to the obtainment of the results graphically expressed in figure 1.
From the graph presented above we can see a rapid increase in the total aflatoxin content in the range 15 -19 C followed by a slow increase in the range of 19-22 C and again an important raise up to 25 C reaching the limit of acceptability of 4ppbIncrease the maximum total aflatoxin is done in the range 15-19 ^oC due to favor moulds producing mycotoxins

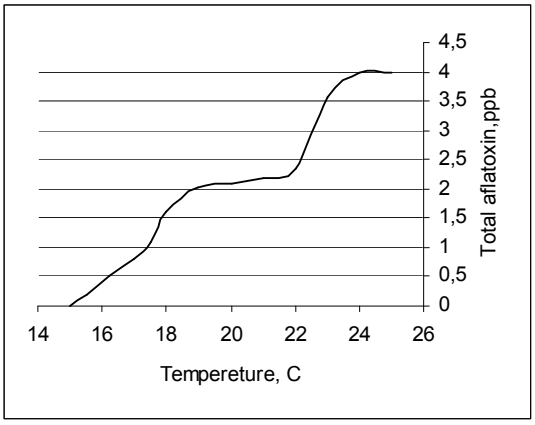

Figure 1: Total aflatoxin content from the wheat samples taken from the horizontal storehouses

Using statistical analysis we determined the value of the correlation coefficient between temperature variation and total aflatoxin content and we obtained a correlation coefficient value equal to 0.98. We therefore conclude that there is a strong correlation between variation in the same sense between temperature variation and total aflatoxin content.

The determination of the B_1 aflatoxin of the wheat samples taken from horizontal warehouses led to the obtainment of the results expressed in figure 2.

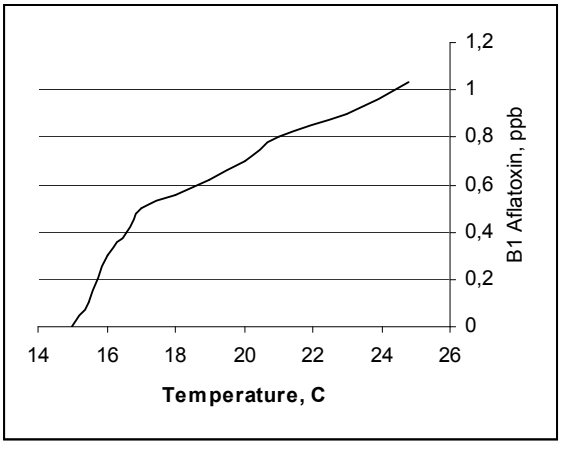

Figure 2:B1 aflatoxin content from the wheat samples taken from the horizontal storehouses

From the graph presented above we can see that the B_1 aflatoxin has a pronounced increasing rate starting from 15˚C up to 17˚C, after which it has a a medium increase until at over 25˚C . We note that the 25 ^{o}C value temeperatura of aflatoxin B1 is 1 ppb below the 2 ppb as the maximum limit established for cereals for human consumption [9].

Calculation of the coefficient of correlation between temperature variation and aflatoxin B1 content led to obtaining a correlation coefficient value of 0.95. So in the case of aflatoxins B1 have the same effect, a strong correlation between temperature variation and aflatoxin B1 content.

4. CONCLUSIONS

Following the carried out experimental determinations we have reached the conclusion that:

The use of low temperature leads to the cessation mould forming, mould that is potentially toxicogenic in mycotoxins, for all the samples tested where the wheat's temperature was under 14˚C, the presence of mycotoxins was undetectable.

The presence of mycotoxins in low quantities, was detected above the 15˚C temperature.

The presence of mycotoxins close or over the admissibility limit according to the European norms was detected at a temperature higher than 24˚C.

Statistical analysis revealed a strong correlation between temperature variations in the same sense and content of total aflatoxin and aflatoxin B1, which recommends keeping the grain at low temperatures as an effective method of reducing the content of mycotoxins in cereals for human consumption.

As a result it would be desirable that all companies in the storage and preservation of cereal field, should use the cereal cold preservation technology.

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