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## Fungus and mycotoxins in wheat grain at post harvest

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### Abstract

The aim of this work was to assess the fungus and mycotoxins contamination in wheat grain (*Triticum aestivum*) in post harvest during 180 days of storage. Five samples were taken at 30 days intervals during the six months of storage period, assessing fungus such as: *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., and mycotoxins such as aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub> and AFG<sub>1</sub>, AFG<sub>2</sub>), ochratoxin A (OTA), zearalenone (ZON), deoxinivalenol (DON) and fumonisins (FB<sub>1</sub> and FB<sub>2</sub>). The results showed that in all 35 samples analyzed many mycotoxins such as aflatoxins, ochratoxin A, zearalenone, deoxinivalenol and fumonisin FB<sub>2</sub> were detected and the fumonisin FB<sub>1</sub> were present in 11 samples (31.4 %) with levels of 36.3 to 2,891 mg/g. The presence of the *Aspergillus* spp., fungus showed in 100 % of the samples, the *Fusarium* spp. in 80 %, and *Penicillium* spp. in 60 % of the samples. The influence of grain fumigation during the storage period on the microorganism development, by reducing the percentage of fungus in the grain bulk, was also verified. This was due to the deleterious effect of phosphine on fungus development. As the mycotoxins can not be removed from the grain during milling process it is important to prevent their development by

reducing the temperature and moisture content, and also the pest contamination in the bulk grain.

*Key words:* Mycotoxins, storage grain, fungus, wheat quality.

### Introduction

Cereal grain can be contaminated by a great variety of microscopic fungus during its development. These pathogens may affect the plant resulting in a reduction of the grain quality; some species can even release natural toxins that cause human and animal intoxication. Mycotoxins prevention is very important as, once developed, they become stable at environment temperature and very resistant to thermal changes (Scudamore, 2005).

Mycotoxins are a cause of concern in grain storage, being one of the many important factors, as they come from fungus development due to previously existent conditions such as: moisture content, temperature, storage period, contamination rate, broken grain and impurities, insect presence, oxygen rate, damages during harvest processing and grain and seed transport (Lazzari, 1997; Scussel, 2002; Santos, 2002; Garcia et al., 2003; Scudamore, 2005).

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The main mycotoxins classes that occur in cereal are the aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub> and AFG<sub>1</sub>, G<sub>2</sub>), the tricotecens, deoxinivalenol (DON) and (T-2 toxin), the fumonisins (FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>), the zearalenone (ZON), ochratoxin A (OTA) and the ergot alkaloids, the majority of the mycotoxins in these groups are produced by three fungi genus: *Aspergillus*, *Penicillium* and *Fusarium* (Council for Agricultural Science and Technology, 2003). The species of the *Aspergillus* and *Penicillium* genus are the ones that proliferate easier in stored grain (Puzzi, 1986). Some fungus develop in different temperatures and produce toxins in really low temperatures such as the ones from the *Fusarium* spp. genus, this is one of the reasons why in some Brazilian regions the winter crop grain have different toxins from those produced during the warm seasons (Krabbe, 1995).

The use of phosphine in the control of *Aspergillus flavus* on stored maize, by maize grain fumigation under different temperatures with concentrations equal or superior to 3 gm<sup>-3</sup>, was able to control the growth and development of the *A. flavus* "in vitro", but was not able to control it's growth and development in maize grain when submitted to the same dose of phosphine (3 gm<sup>-3</sup>), in an exposure period of 120 hours (Da Mata et al., 1999). Phosphine has the capacity to delay de development of fungus in stored seeds with humidity content above the recommended for safe storage (Agarwal and Sinclair, 1997).

Besides fungus, another problem is insect infestation which causes damage to the grain tegument and produces carbonic acid and water, contributing to the increase of the humidity content which then causes an increase in the grain respiration and consequentially in the temperature. This scenario favors fungus multiplication. Also, insects due to their movement help to disseminate the spores of fungus all over the grain bulk (Santos and Mantovani, 1997).

The contamination by AFLs was assessed in four samples of maize, separated according to the Brazilian classification standards, and it was verified that the fraction that contained decomposed, burnt and sprouting grain normally had the highest levels of aflatoxins (Gloria et al., 2004).

Using commercial (special, common and whole meal) wheat flour from the southern region of Rio Grande do Sul, it was verified that the physical-chemical analysis, such as the high ash levels, can explain the presence of mycotoxins in the flours (Vieira et al., 1999). OTA and DON were detected in levels of 12 and 53µg/kg respectively. On wheat flours, the presence of OTA leads to the suspicion of contamination by the T-2 and AFB<sub>1</sub> toxins also (Badiale-Furlong et al., 2003). The wheat and it's byproducts quality (common and special flour) were analyzed at the laboratory of Cotriguaçu Mills, the wheat was stored dry with 13 % humidity and conditioned (humidified) with 15 % moisture content. It was verified that there was no presence of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, OTA and DON toxins, however for FB<sub>1</sub> the levels varied between 0.5 and 3.9 µg/kg for wheat and between 0.6 and 2.3 µg/kg for the flours ( Birck et al., 2003).

DON is a toxic fungus metabolic produced by species from the *Fusarium* genus, belonging to the tricotecens type "B" group. This mycotoxin can contaminate fruits, seeds and byproducts, moreover wheat flour is a substrate very vulnerable to DON contamination. In studies with the analysis of 78 wheat flour samples, 27 (34.61 %) showed contamination by DON with a mean and maximum levels of 283.94 g/kg and 794 µg/kg, respectively (Araujo et al., 2004). However, the contamination by DON in wheat (297 samples) from the southern region was of 74 (24.91 %) with a mean and maximum levels of 603.2 µg/kg 8,504 g/kg (Mallmann et al., 2003). DON intoxication can decrease hematopoiesis by damages to the bone marrow and cause damage to the nervous, gastrointestinal and cardiovascular systems.

Studies done in India as to the presence of mycotoxins (AFLs and tricotecens) in 150 samples of sorghum, maize, wheat, refined whole meal wheat flour and animal food samples mixed, showed aflatoxins AFB<sub>1</sub> (20-60 mg/kg) levels in 4 sorghum samples (Ramakrishna et al., 1990). Of the 64 sorghum samples, harvested in agricultural areas of Georgia, it was verified the incidence of ZON in levels of 2 to 1,468 mg/kg in 31 % of the samples, furthermore, 35 % of the

samples did not reject the possibility of mycotoxin (AFLs) formation in grain still on the farm (McMillian et al., 1983). Analysis of 140 sorghum samples, just harvested and stored, identified presence of FBs in 74.2 % of the samples with levels between 0.11 and 0.15 µg/g (Da Silva et al., 2003).

Due to the increasing pressure from the intern and extern consumer markets as to the product's quality standards, this study had the objective of assessing the wheat, coming from commercial farms and stored wheat, contamination as to the fungus and mycotoxin production so as to offer technical advise to the storage and processing sectors (Milling Industries).

## Material and methods

For the experiment, 1,000 tons of grain of the Coodetec 104 (74 %), Iapar 78 (25 %) and Iapar 85 (1 %) varieties, produced in the western region of Paraná, were used. After being harvested, the wheat was submitted to drying (13 % moisture content) and to the cleaning standards of 1 % maximum impurities. The sampling was carried out according to the official method of the MAPA, every 30 days for a period of 180 days (Brasil, 2001). The 1,000 tons used in the experiment were stored in a concrete vertical silo with 18.0 m of height and 14.8 m diameter, virtually divided in 5 equal parts (A, B, C, D and E), totaling 5 samples per period (1 day, 30 days, 60 days, 90 days, 120 days, 150 days and 180 days), in a total of 35 samples collected during the experiment. For each sampling point 1.0 kg of wheat grain was taken to be analyzed as fungus and mycotoxins contamination.

## Results and discussion

### Fungus contamination

From the five samples of wheat grain recently harvested, the results showed fungus contamination by the *Aspergillus* spp. in four samples, in four

samples by *Fusarium* spp. and three samples by the *Penicillium* spp. The remaining 30 samples, corresponding to the 180 days storage period, 29 samples (96.7 %) showed fungus contamination by *Aspergillus* spp., 14 samples (46.7 %) by *Fusarium* spp. and 24 samples (80.0 %) by *Penicillium* spp. (Table 1). The fungus from the *Fusarium* spp., specifically the *F. graminearum* and *F. verticillioides* were more commonly found in maize and its byproducts, as reported by Scaff, (2003).

It was observed that, after-harvest (1 day) and storage (30 days), the fungus of the *Fusarium* spp. showed the highest UFC/g values, which decreased gradually until the end of the storage period showing the highest UFC/g growth. There was a reduction in the fungus growth of the *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. in the phosphine treatment period at the end of 30 and 150 days of storage, evidenced by the samples of 60 and 180 days of storage.

For the *Aspergillus* spp. the UFC/g levels were higher just after harvest (1 day) and after 30 days of storage, decreasing gradually along storage time, and the lowest UFC/g values were at 60 and 120 days of storage. The greatest incidence of fungus growth occurred in the middle of the silo, where it was observed that the *Aspergillus* spp. Fungus belonged in greater quantity to the *niger* group. The fungus *Penicillium* spp. showed the highest level of UFC/g in the storage period of 90 and 150 days, and lowest UFC/g in the period just after harvest (1 day) and 60 days of storage.

The fungus *Fusarium verticillioides* was the most frequent followed by the *Penicillium* spp., *Aspergillus* spp. and other 10 filamentous fungi genus, on grain with moisture content of 12.3 to 17.8 %, mean temperature of 18.4 to 24.1 °C, pluvial precipitation of up to 337 mm and relative humidity between 64 and 87.8 %. The mycotoxicological analyses did not show mycotoxin presence (Pozzi and Corrêa, 1994).

Some fungus, such as those of the *Fusarium* spp. shows a greater toxin production with temperatures between 10 and 15 °C. However, most fungus prefers temperatures between 25 and 28 °C (Krabbe, 1995).

**Table 1.** Total number of filamentous fungus and yeasts (UFC/g) in post-harvest wheat during the period of November 2003 to May 2004.

Storage Period (day)	Sample													
	<i>Aspergillus</i> spp.							<i>Fusarium</i> spp.						
	Total	Mean	A	B	C	D	E	Total	Mean	A	B	C	D	E
1	807	161.4	53	200	350	120	84	39	7.8	3	12	20	-	40
30	1092	218.4	42	130	800	100	20	57	11.4	-	15	34	-	8
60	26	5.2	3	18	-	2	3	10	2	2	-	8	-	-
90	173	34.6	100	36	25	8	4	6	1.2	1	-	3	-	2
120	54	10.8	28	9	4	10	3	12	2.4	-	7	-	5	-
150	685	137	100	280	157	93	55	5	1	3	-	-	2	-
180	376	75.2	160	73	46	68	29	3	0.6	-	1	-	2	-

  

Storage Period (day)	Sample													
	<i>Penicillium</i> spp.							Yeast						
	Total	Mean	A	B	C	D	E	Total	A	B	C	D	E	
1	8	1.6	-	-	6	1	1	28	-	12	1	1	14	
30	22	4.4	-	15	4	-	3	1	-	1	-	-	-	
60	3	0.6	-	2	-	-	1	3	1	-	-	-	2	
90	81	16.2	2	10	22	31	16	-	1	-	-	-	-	
120	65	13	-	35	22	6	2	1	-	-	1	-	-	
150	127	25.4	7	13	61	42	4	1	-	-	1	-	-	
180	69	13.8	8	36	18	2	5	2	-	-	2	-	-	

(-) Absence of fungus.

Many of the samples contaminated by fungus of *Aspergillus* spp. were also contaminated by insects (Da Mata et al., 1999). The association between *Aspergillus flavus* and stored grain insects has drawn the attention of many authors. A population of insects in the grain bulk if not controlled may create local temperature and humidity conditions that stimulate the fast development of fungus, which may lead to the grain bulk deterioration and mycotoxin production.

During the storage period, at 60 and 180 days of storage, a phosphine treatment was done so as to eliminate the insect infestation, it was observed that there was a reduction in the number of Colony Forming Units UFC/g and in the diameter of the colonies, which continued to

grow little harmed. Phosphine has the capacity to delay the fungus development, this delay occurs in stored seeds with a moisture content above the recommended for safe storage (Agarwal and Sinclair, 1997). In our experiment, there was a reduction with low moisture content (Table 2), as the maximum recommended humidity level according to the Normative Instruction N°. 7 is of 13 % (Brasil, 2001).

*In vitro* phosphine was efficient in the control of fungus in doses equal or superior to 3 g/m<sup>3</sup> (Da Mata et al., 1999). These authors also verified that in grain the dose of 3 g/m<sup>3</sup> for an exposure period of 120 hours was not effective in the control of fungus under different temperature and that the fumigant did not control the fungus in the intrinsic part of the maize grain.

**Table 2.** Mycotoxin contamination, moisture content and water activity in stored wheat in the period of 180 days.

Day	Sampling point	Moisture Content (%)	Water Activity (aw)	Mycotoxin ( $\mu\text{g}/\text{kg}$ )								
				AFB <sub>1</sub> <sup>a</sup>	AFB <sub>2</sub> <sup>b</sup>	AFG <sub>1</sub> <sup>c</sup>	AFG <sub>2</sub> <sup>d</sup>	OTA <sup>e</sup>	ZON <sup>f</sup>	DON <sup>g</sup>	FB <sub>1</sub> <sup>h</sup>	FB <sub>2</sub> <sup>i</sup>
1	A	11.0	0.631	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	11.2	0.634	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	11.8	0.636	ND	ND	ND	ND	ND	ND	ND	53.8	ND
	D	11.1	0.625	ND	ND	ND	ND	ND	ND	ND	36.3	ND
	E	10.6	0.639	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	A	11.5	0.627	ND	ND	ND	ND	ND	ND	ND	100	ND
	B	11.6	0.621	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	11.4	0.647	ND	ND	ND	ND	ND	ND	ND	ND	ND
	D	11.1	0.625	ND	ND	ND	ND	ND	ND	ND	ND	ND
	E	11.5	0.621	ND	ND	ND	ND	ND	ND	ND	200	ND
60	A	11.7	0.592	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	11.7	0.603	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	11.9	0.603	ND	ND	ND	ND	ND	ND	ND	38.1	ND
	D	11.8	0.613	ND	ND	ND	ND	ND	ND	ND	ND	ND
	E	12.0	0.601	ND	ND	ND	ND	ND	ND	ND	51.1	ND
90	A	10.5	0.602	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	10.6	0.594	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	11.0	0.588	ND	ND	ND	ND	ND	ND	ND	ND	ND
	D	11.1	0.587	ND	ND	ND	ND	ND	ND	ND	ND	ND
	E	11.0	0.588	ND	ND	ND	ND	ND	ND	ND	ND	ND
120	A	10.6	0.596	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	10.8	0.592	ND	ND	ND	ND	ND	ND	ND	148	ND
	C	10.8	0.595	ND	ND	ND	ND	ND	ND	ND	ND	ND
	D	10.9	0.606	ND	ND	ND	ND	ND	ND	ND	86.5	ND
	E	10.6	0.602	ND	ND	ND	ND	ND	ND	ND	2891	ND
150	A	11.7	0.608	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	11.8	0.681	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	11.5	0.679	ND	ND	ND	ND	ND	ND	ND	ND	ND
	D	11.5	0.688	ND	ND	ND	ND	ND	ND	ND	ND	ND
	E	11.6	0.689	ND	ND	ND	ND	ND	ND	ND	ND	ND
180	A	12.4	0.673	ND	ND	ND	ND	ND	ND	ND	53.2	ND
	B	12.4	0.673	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	12.3	0.681	ND	ND	ND	ND	ND	ND	ND	ND	ND
	D	12.7	0.685	ND	ND	ND	ND	ND	ND	ND	54.7	ND
	E	12.2	0.682	ND	ND	ND	ND	ND	ND	ND	ND	ND

<sup>a</sup>Aflatoxin B1, <sup>b</sup>Aflatoxin B2, <sup>c</sup>Aflatoxin G1, <sup>d</sup>Aflatoxin G2, <sup>e</sup>Ochratoxin A, <sup>f</sup>Zearalenone, <sup>g</sup>Deoxinivalenol, <sup>h</sup>Fumonisin B, <sup>i</sup>Fumonisin B, ND = Not detected by the used methodology.

## **Moisture content**

Correlations between the grain and flour's moisture content and the water activity during the storage period were observed, the mean initial moisture content was of 11.1 % raising to 12.4 % during 180 days of storage, the initial water activity was of 0.63 % raising to 0.68 %, it was observed that there was a gradual increase of moisture and water activity (Aa) from 150 to 180 days of storage. It was also verified that there is a correlation between the fungus increase in the period of 120 days to the period of 150 – 180 days (Table 1) with the water activity and insect infestation (Table 2). The water activity increase may be related with the presence or not of insect infestation, as it was observed that there was an infestation in this period. The presence of insects, besides causing grain damage and disseminating contamination, increases the water content and the grain temperature due to its movements (Scudamore, 2005; Santos, 2002; Santos and Mantovani, 1997; Lazzari, 1997).

We can conclude that in the moisture content in which the grain was stored, less than 13%, the increase was not significant. However, if the grain was stored with 13 % moisture content with an increase of 1.3 % from the initial period to 180 days ( $13\% + 1.3 = 14.3\%$ ) and with the water activity of 0.68 % with an increase of 0.05 % from the initial period to 180 days ( $0.68 + 0.05 = 0.73\%$ ), the conditions would be ideal for microorganism development. In this manner, we advise that the grain be stored with water activity and moisture content below the recommended for safe storage, to guarantee the grain's integrity.

## **Mycotoxins**

AFLs, ZON, OTA and DON: The quality assessment as to the product contamination by mycotoxins is a factor of great importance in public health because of the risk it represents. The 35 wheat grain samples during the whole experiment didn't show positive results for AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, ZON, OTA and DON contamination (Table 2). This can be due to the

moisture content and water activity, which were not very favorable to fungus development and mycotoxin production. The mycotoxins present in wheat flour may not be necessarily due to wheat contamination, but to contamination during the milling processing or in conditioned wheat (humidified with 15 to 16 %), and product shelf storage time and condition (Vieira et al., 1999).

Fumonisin: Of the 35 samples analyzed 11 (31.4 %) showed FB<sub>1</sub> contamination and none showed FB<sub>2</sub> contamination. FB<sub>1</sub> contamination occurred in the initial period (1 day) and after 30, 60, 120 and 180 days of storage. In the period from 90 to 150 days of storage there was no contamination in any samples of the silo. For FB<sub>2</sub> there was no contamination in any period during grain storage (Table 2).

Assessing wheat grain and flour quality after processing at Cotriguaçu Mills, fumonisin levels between 0.5 and 3.9 µg/g were found for wheat grain and 0.6 to 2.3 µg/g for wheat flour (Birck et al., 2003). This data shows that fumonisin is not eliminated during grain cleaning and milling processes. It is equally distributed in wheat flour and byproducts during the milling process.

Traceability is fundamental to help in the control measures and to guarantee the safety of the food, free of natural and chemical contaminants. To better correlate the results obtained in this study and to determine preventive control measures, the chemical products (pesticides) as well as the environment conditions (temperature, relative humidity and pluvial precipitation) were traced.

Considering that toxic fungus and FB<sub>1</sub> toxins were detected and that one of the microbiological problems of the milling industries is the high levels of mould and ferments, the adoption of control measures in all the production chain is vital, from the crop production to the harvest and storage and also through the processing of wheat byproducts.

Mycotoxins (AFLs, OTA, ZON e DON) were not detected in any of the 35 wheat samples analyzed during the storage period, which means a good sanitary and storage condition.

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