**Abstract**

Food safety and hygiene are the main statements of the present and future of grain and food production in the world. Grain losses are common, especially for wheat, due to production problems as environmental conditions, diseases, pests, harvest machinery, loading, drying and post harvest conservation. Contaminants, pesticides residues and mycotoxins can cause health problems to human beings due to food consumption of grain products. With the aim of preserving the wheat quality from the harvesting to industrialization the Hazard Analysis and Critical Control Points (HACCP) was implemented at the wheat production chain associated to stored grain unit which belongs to the Cooperativa Integrada, located at Assaí in the State of Paraná, Brazil. The HACCP methodology was developed collecting data from cultural and harvest processes, wheat grain transportation to silos, drying process, pesticide treatment of grain and storage parameters. Also several analysis from wheat grain sampled periodically at reception of grain at stored unit and during storage period were performed looking for contaminants as insect pests, insect fragments, mycotoxins, and the technological quality of wheat grain. The results showed three critical control points (CCP), related to mycotoxins, at the steps of reception, drying and storage of grains. This shows the need to implement the Hazard Analysis and Critical Control Points (HACCP) Program that associated with Good Agricultural Practices (GAP) and Integrated Pest Management (IPM) should assure the safety of stored wheat.

**Key words**: wheat, mycotoxins, food safety, HACCP.

**Introduction**

The Hazard Analysis and Critical Control Points (HACCP) was created in the United States of America in 1959 and has been recognized in Brazil by official institutions as Ministry of Agriculture, Ministry of Health and Ministry of Science and Technology. This recognition assures the introduction of certified agricultural products within the internal market and increases the competitively at international level, which could supplant possible non chargeable boundaries (CampoPAS, 2004).

According to FAO (Food and Agricultural Organization of the United Nations), HACCP system is a preventive and systematic approach.
aiming at biological, chemical and physical hazards through prevention instead of inspection and tests in final products (INPPAZ-OPAS-OMS, 2001).

The Brazilian primary production had showed limitations concerning physical, chemical and biological hazard control, mainly because the pre and post harvest stages need more care, which could lead to diseases transmitted by foods (CampoPAS, 2004).

Wheat is one of the most important grains to humanity. The main wheat producing countries are: China, USA, India, Canada and Russia. According to Conab’s (2006) data, Brazil produced around 5.8 million ton of wheat in the 2003/04 and 2004/05 crops, and it has been predicted for the 2005/06 crop a production of 4.8 million ton which represents around 50 % of the national demand.

The grading of wheat is based on physical characteristics, such as: test weight, foreign materials, damaged kernels (heat-damaged, insect-damaged, mold-damaged), shrunken and broken kernels and dockage. The presence of live insects in a wheat load disqualifies it and makes its commercialization not allowed (Normative Instruction n° 7, August 15, 2001, Brazilian Ministry of Agriculture).

The growing need for products to make up for the world food demand, bearing in mind the population growth, requires that the quality of the grain obtained from the harvest is kept with the minimum loss possible until final consumption (Lorini, 2003). According to Caldas (1999), every year, a bumper quantity of wheat is lost due to environmental problems, diseases such as fusarium ear blight (FEB), besides inadequate operations at harvesting, drying, transportation and storage.

Mycotoxins are fungi metabolites that are toxic when consumed by humans or animals. They are hazard examples which could appear in the wheat grain and consequently in its derivatives. The FEB of wheat, caused by Gibberella zeae (anamorph Fusarium graminearum), happen in every region that produces this cereal. For many years it has been considered a secondary illness, however, due to the growth of its frequency and its severity, it has appeared as one of the main diseases of wheat. The main toxins produced by Fusarium graminearum are: Zearelonone (ZEN), Deoxynivalenol (DON), Nivalenol (NIV t), Fusarenon-x (FUS-X), Toxin T2 and other trichothecenes (Lianos, 2005). Furlong et al. (1995a,b) reported the occurrence of mycotoxin of Fusarium spp. in wheat produced in São Paulo state, in the south of Brazil, Argentina and Uruguay.

Aflatoxins can be found in food contaminated by the fungus Aspergillus flavus and Aspergillus parasiticus. These mycotoxins are produced by storage fungi which need more than 13.0% of moisture content in the grains and are associated with the presence of insects. Some preventive actions could control the aflatoxin level, such as fast drying and the storage under controlled conditions of relative humidity (Balbani and Butugan, 2001).

Other hazard in wheat is represented by pests. The medium quantitative losses caused by pests in Brazil, estimated by the FAO and by the Brazilian Ministry of Agriculture, are around 10,0 % from the total produced annually (Lorini, 2003).

Among the agro toxics the chemical products used in the wheat storage are the most problematic. They are from two groups: the pyrethroids, which can leave residues in the flour (Skerritt et al.,1996) and the organophosphorades, which although they are much more toxic to human beings at the application moment, they remain less time in the wheat and leave less residues than the pyrethroids (Papadopoulou-Mourkidou and Tomazou,1991; Skerritt et al., 1996).

This work was based in Good Agricultural Practices and focuses the principles of HACCP. It aimed at making technological solutions feasible, with the creation of tools and rules to the preservation of the food identity and assurance of safety of wheat produced and stored at Cooperativa Integrada located at Assai, in the State of Paraná, Brazil.
Material and methods

The work was developed at the storage unit which belongs to Cooperativa Agropecuária de Produção Integrada do Paraná (Cooperativa Integrada) – located at Assai – in the state of Paraná, Brazil during the 2005 crop. The action area from the Assai unit comprises some cities from the North of Paraná, such as: Assai, São Sebastião da Amoreira, Santa Cecília do Pavão, Nova Santa Bárbara, São Jerônimo da Serra, Santo Antonio do Paraíso, Jataizinho and Uraí. The static capacity for storage of wheat is 25,200 ton, distributed in several bins.

The complete pre / pos-harvest process was divided in three steps: production, transportation and storage. In each phase it was done the diagnostic through data collection and sampling and, after processing of data, they were determined the critical points.

Production: It was developed a following program, through questionnaire application ran by the Technical Team, to 20 % of the producers, chosen within the action area. Data about cultivar selection, environmental conditions, culture rotation, fertilizers, diseases and pest attacks, management of fungicides, insecticides and herbicides and harvest were collected

Transportation: They were observed the hygienic conditions of vehicles used to transport the grains from farm to the storage unit (residues of chemical products, waste of other cultures and pests).

Storage: This step was considered from the arrival of the trucks at the Unit until the load out of product clean and dry. Many spreadsheets were developed and applied throughout the process of storage.

Samples were collected according with the flux of grain in the Unit. At the reception, once a weak during harvesting, a composite sample was obtained from the trucks incoming in a day. Sampling plan and analysis (moisture, test weight and damaged grains) meet the stipulations of the Brazilian Ministry of Agriculture (Normative Instruction n° 7, August 15, 2001) (MAA, 2001). The incidence of fungi was evaluated through Blotter test (Brasil, 1992).

The wheat received was stored in different bins. Composite samples were collected after drying, representing loading of each bin and also after three month storage, corresponding to unloading of bins. These samples were submitted to the same analysis described before and also to mycotoxins determination: Zearalenone, by Thin Layer Chromatography (TLC), according official method n. 976.22 – AOAC (1990) and DON by TLC, according official method n. 986017 - AOAC (1995).

Results

The environmental conditions for planting and harvest of wheat in the 2005/crop, was extremely unfavorable. The recommended time to plant in the Assai region extends from 21 March to 20 June. Throughout this period, the availability of water to plant (above 50 mm within three days) only occurred from May 22 and 23, which led to a delay in the culture implementation and/or implementation in dry soil conditions.

During the crop development and especially during flowering, which happened from July 5 to August 15, it was registered rain only in July 16 (7.0 mm), July 24 (14.0 mm) and August 8 (2.0 mm), as it is showed in Figure 1.

It was observed that the biggest amplitude of temperature happened on July 05 and 25. These days coincide with flowering for the major part of the production fields.

The Assai Unit received 18,953 ton of wheat which were delivered by the producers in 2,124 loads or vehicles and were classified at the reception according to the Regulations and Procedures established. The averages of moisture and test weight of wheat were 18.1 % and 78 %, respectively. The impurities had a medium discount of 2.8 %. The average time of trucks waiting to load was 1 hour 43 minutes, corresponding to a loading of 4.6 ton/hour. Around 5 % of the production remained on the unload line for more than 6 hours, being 11 hours the maximum time registered. The drying to 13.0 % moisture was done immediately after the unload.
The application and following of Regulations, Procedures and Registers established allowed verifying that the hazard represented by pests and chemical residues was correctly being controlled with the Integrated Pest Management (IPM) and the Good Agricultural Practice (GAP) already implemented at the Unit.

The wheat cultivars received were: CD-104 (59.58 %), Iapar-78 (5.55 %), Alcover (3.65 %), BRS-208 (3.47 %), CD-111 (2.30 %), Taurum (1.74 %), BRS-210 (1.17 %), BRS-220 (1.04 %), other (< 1.00 % each) and without information (20.11 %). Although none of these cultivars are considered resistant to *Giberella*, by the Brazilian Center South Wheat and Triticale Research Commission (Embrapa, 2004, 2005), visual analysis of grains showed infection (1 to 4 scabs grains in 50 g sample), in only 2.2 % of the total amount of wheat crop. Then, the environmental conditions during crop development were not propitious to the attack of these fungi.

In the microbiological analysis in the samples at reception, after drying and after storage, it was observed the presence of *Fusarium* spp, *Aspergillus* spp, *Penicillium* spp and *Alternaria* spp, with the predominance of the latest (Table 1).

On Table 2 it is presented the mycotoxins results. The DON content varied from 0.09 to 0.299 ppm, and zearalenone was not detected.

**Discussion**

During the three months after culture implementation it was observed low rate of precipitation, which is normal in this area and contributes to low incidence of fungi or bacterial diseases. The rain came back in September and October, during harvest time.

The general condition of grains in the storage and the joint of physical, chemical, biological and sensorial characteristics established at the harvest define the ability of the grain in resist the attacks of insects and microorganisms. During harvesting, drying and elevator transportation, the grains are subject to mechanical impact which could lead to breaks, which would be an open door to fungi and insects. Dry and cold grains can best keep the original quality of the product. The moisture content is considered the most important factor in the storage grain deterioration process control (Regitano-D’Arce, 1995).

The storage fungi demand relatively low moisture content to survive, for instance, *Aspergillus restrictus* needs a minimum of 13.5 % moisture in the grain, *Aspergillus glaucus* 14.0 %,
Table 1. Results from the analysis of Blotter Test

<table>
<thead>
<tr>
<th>Harvest Period</th>
<th>Place of storage</th>
<th>Sampling Points</th>
<th>Fusarium spp (%)</th>
<th>Alternaria spp (%)</th>
<th>Penicillium spp (%)</th>
<th>Aspergillus spp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/09/05</td>
<td>- x</td>
<td>0.0</td>
<td>73.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>08/08 a 15/09</td>
<td>Bin I</td>
<td>x</td>
<td>12.0</td>
<td>20.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>08/08 a 15/09</td>
<td>Bin I</td>
<td>x</td>
<td>1.0</td>
<td>3.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>17/09/05</td>
<td>- x</td>
<td>4.0</td>
<td>83.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>20/09/05</td>
<td>- x</td>
<td>3.0</td>
<td>88.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>16/09 a 28/09</td>
<td>Bin I</td>
<td>x</td>
<td>7.0</td>
<td>34.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>16/09 a 28/09</td>
<td>Bin I</td>
<td>x</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>28/09/05</td>
<td>- x</td>
<td>0.0</td>
<td>85.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>29/09 a 30/09</td>
<td>Bin II</td>
<td>x</td>
<td>9.0</td>
<td>34.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>29/09 a 30/09</td>
<td>Bin II</td>
<td>x</td>
<td>0.0</td>
<td>3.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>04/10/05</td>
<td>- x</td>
<td>7.0</td>
<td>76.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>30/09 a 03/10</td>
<td>Bin III</td>
<td>x</td>
<td>6.0</td>
<td>32.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30/09 a 03/10</td>
<td>Bin III</td>
<td>x</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>11/10/05</td>
<td>- x</td>
<td>5.0</td>
<td>83.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>04/10 a 20/10</td>
<td>Bin IV</td>
<td>x</td>
<td>6.0</td>
<td>54.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>04/10 a 20/10</td>
<td>Bin IV</td>
<td>x</td>
<td>5.0</td>
<td>1.0</td>
<td>0.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1 Blotter test with Deep Freezing and cleaning.
R=Reception. Composite sampling at the trucks.
AD = After drying. Composite sampling at the bins loading.
AS = After storage (3 month). Composite sampling at the bins unloading.

Table 2. Results from the analysis of mycotoxins in different samples of wheat.

<table>
<thead>
<tr>
<th>Harvest Period</th>
<th>Place of storage</th>
<th>Sampling Points</th>
<th>DON (ppm)</th>
<th>Zearalenone (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/08 a 15/08</td>
<td>Bin I</td>
<td>x</td>
<td>0.299</td>
<td>Negative</td>
</tr>
<tr>
<td>08/08 a 15/08</td>
<td>Bin I</td>
<td>x</td>
<td>0.266</td>
<td>Negative</td>
</tr>
<tr>
<td>16/09 a 28/09</td>
<td>Bin I</td>
<td>x</td>
<td>&lt;0.090</td>
<td>Negative</td>
</tr>
<tr>
<td>16/09 a 28/09</td>
<td>Bin I</td>
<td>x</td>
<td>&lt;0.090</td>
<td>Negative</td>
</tr>
<tr>
<td>29/09 a 30/09</td>
<td>Bin II</td>
<td>x</td>
<td>0.120</td>
<td>Negative</td>
</tr>
<tr>
<td>29/09 a 30/09</td>
<td>Bin II</td>
<td>x</td>
<td>0.105</td>
<td>Negative</td>
</tr>
<tr>
<td>30/09 a 03/10</td>
<td>Bin III</td>
<td>x</td>
<td>0.120</td>
<td>Negative</td>
</tr>
<tr>
<td>30/09 a 03/10</td>
<td>Bin III</td>
<td>x</td>
<td>0.105</td>
<td>Negative</td>
</tr>
<tr>
<td>04/10 a 20/10</td>
<td>Bin IV</td>
<td>x</td>
<td>&lt;0.090</td>
<td>Negative</td>
</tr>
<tr>
<td>04/10 a 20/10</td>
<td>Bin IV</td>
<td>x</td>
<td>0.135</td>
<td>Negative</td>
</tr>
</tbody>
</table>

AD = After drying. Composite sampling at the bins loading.
AS = After storage (3 month). Composite sampling at the bins unloading.

Aspergillus candidus 15.0 %, Aspergillus ochraceus 15.0 %, Aspergillus flavus 18.0 % and Penicillium spp from 16.5 % to 19.0 % (Gwinner et al., 1997). Although the average moisture content of wheat at reception was 18.1 %, which is excellent for storage fungi development, this was not observed (Table 1), due to agility in the unload and drying of
the product.

Among the factors which could affect wheat production, are the occurrence and the intensity of diseases (Reis et al., 1996). *Gibberella zeae* was observed causing damage in wheat producing regions where the weather is humid and hot, with high rain precipitation (more than 48 hours of wetness) in the flowering phase of the wheat (Andersen, 1948; Sutton, 1982; Reis, 1990). The gibberela ear blight intensity is highly dependent on the environmental conditions to its establishment; therefore it varied year by year.

The results observed (scabs grains percentage, Blotter Test and mycotoxins contents) show that, probably, the grain infection could have occurred later, because, even with low rate of damaged grains, there still was a DON mycotoxin production. Analyzing the rain precipitation during flowering it can be observed the increase of relative humidity, on July 24 and 25 (Figure 1), which could have promoted the infection by *G. zeae* in plants on the late flowering level.

In his work about gibberela in wheat, Del Ponte (2004) verified that grains contaminated with toxins from late inoculations, presented similar weigh to the control grains (without inoculation), showing that late contamination can cause impact only in the quality of the grain and not in its productivity.

Although the level of mycotoxin DON registered was below what is accepted by the World Health Organization and established by the United States and Canada (maximum 2.0 ppm in grains), it has been noticed that the following and register of the environmental conditions during the development of culture and the fast evaluation of mycotoxins during the reception, could help the management of the storage and commercialization. Zearalenone practically have not been found, perhaps due to environmental conditions in the field.

**Conclusion**

Since GAP and IPM programs are adequately implemented at the Assai Unit, only mycotoxins remains as a hazard needing control. Three CCP (critical control point) related mycotoxins were detected: reception, drying and storage. Although results showed that in the North of Paraná the environmental conditions during 2005 cropping season do not allowed great *G. zeae* proliferation it was observed some DON production, suggesting that at reception quick test for mycotoxins could be applied, especially when there are suspicion of contamination in the field.

Drying, the second CCP, is the step responsible for the conservation of the product during storage, not giving any conditions to development of storage fungi and consequently mycotoxin production. The storage itself, must be constantly monitored through sampling and thermometry to management of optimal conditions of temperature and storage moisture content.

**Acknowledgements**

Cooperativa Agropecuária de Produção Integrada do Paraná Ltda., Cotriguaçu Cooperativa Central, LCA Ind. e Com. de Produtos Alimentícios Ltda., ESALQ, CNPQ.

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