An overview of the applications of hazards analysis and critical control point (HACCP) system to mycotoxins

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Abstract: Mycotoxins are toxic metabolites produced by fungal species that commonly contaminate staple foods and feeds. The worldwide mycotoxin contamination of food and feed is a problem of public health as it is shown in the total mycotoxin notifications reported by Rapid Alert System for Food and Feed in the European Union. The inclusion of mycotoxin control in food and feed manufacturing hazard analysis critical control point (HACCP) system is widely used as prevention of mycotoxin occurrence.

Several food commodities are mainly involved in control mycotoxins as cereals, grapes, coffee, nuts, milk and wine manufacturers. Moreover, feed is particularly vulnerable to mycotoxin contamination because typically lower quality ingredients are used and HACCP is used to quality control. Aflatoxins and ochratoxins are the most determined mycotoxins for controlling the quality of the products by rapid tests and liquid chromatography methods.

Despite many years of research, and the introduction of good practices in the food production, storage and distribution chain, mycotoxins continue to be a concern in food safety.

Key words: Mycotoxins, HACCP, food, feed, food safety.

Introduction

Food safety is a term broadly applied to food quality that may adversely affect human health. These include zoonotic diseases and acute and chronic effects of ingesting natural and human-made xenobiotics. Fungus and associated mycotoxins are important factors adversely affecting foods produced using contaminated plant products or animal products derived from animals fed on contaminated feeds (Lee et al., 2001). The worldwide contamination of food and feed with mycotoxins is a significant problem as it is shown in the total mycotoxin notifications reported by Rapid Alert System for Food and Feed of 495 only after pathogenic micro-organisms (European Commission 2015).

Mycotoxins are toxic secondary metabolites of fungi belonging, essentially, to the Aspergillus, Penicillium and Fusarium genera. These naturally occurring chemical compounds can be produced on a wide range of agricultural commodities and under a diverse range of situations worldwide. Significant economic losses are associated with their impact on human health, animal productivity, and both domestic and international trade. There is an ongoing need to protect the health of humans and susceptible animals by limiting their exposure to mycotoxins (FAO, 2001). They affect a wide range of agricultural products, including cereals, dried fruits, nuts, coffee beans and oilseeds, which are the backbone of most developing economies. These major crops are highly susceptible to fungal contamination and mycotoxin production. Mycotoxin contamination of susceptible commodities occurs as a result of environmental conditions in the field as well as improper harvesting, storage and processing operations.

Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects upon the central nervous, cardiovascular and pulmonary systems, and upon the alimentary tract. They may also be carcinogenic, mutagenic, teratogenic and immunosuppressive (Marín et al, 2013).

The HACCP system has been increasingly and successfully applied by the food industry and by official food control authorities to prevent and control risks associated with potential contamination of food products with pathogenic micro-organisms and chemical toxicants. Food safety programs routinely use information about the factors leading to contamination to establish preventive and control procedures, thus providing the consumer with a safe, wholesome food supply (Aiko and Mehta 2015).

The expansion of global market has recalled more and more the attention of the researchers on the presence of mycotoxins in the foodstuff trying to limit health damages fixing more restrictive limits for the presence of mycotoxins in the single food products. Many countries regulate for, or suggest permitted levels of, mycotoxins in foods and feed because of their public health significance and commercial and the governmental agencies concerned make efforts to prevent the presence of mycotoxins; establishment of tolerances and guidelines regarding the analysis and control of these compounds by programs conducted to audit the status of these natural contaminants in foods (Alonzo et al., 2008).

While humans are adapting to cope with environmental changes, such as food scarcity, decreased food quality, mycotoxin regulations, crop production and seasonality, and other climate related modifications, fungal species are also adapting so increased cases of mycotoxin adverse health effects are likely to occur in the future. To guarantee access to quality food for everybody, it is needed a way to balance global mycotoxin standards with the realistic feasibility of reaching them, considering limitations of producers and designing strategies to reduce mycotoxin exposure based on sound research (Marroquín-Cardona et al., 2014).

HACCP has been introduced to promote food safety from farm to table by reducing hazardous biological, chemical and physical agents (Lee et al., 2001). Prevention of food risks means enforcing international regulations on the part of the member states of the World Trade...
Organization, increasing vigilance with regard to illegal imports of food, systematically investigating collective food-borne outbreaks, and finally implementing controls according to the HACCP system (Buissen et al., 2008).

This paper reviews the control of mycotoxin contamination of food and feed in the context of HACCP in economic sectors as feed, cereals, coffee, dried fruits, grapes, milk, nut and wine.

**Techniques for toxin detection and quantification to HACCP purposes**

A number of steps should be taken to minimize mycotoxin contamination worldwide. Within several countries, analytical methods to determine mycotoxin levels for regulatory purposes must fulfill to certain performance criteria. Decisions relating to matters of regulatory or commercial arbitration need to be based on agreed and well defined methods of analysis, which are normally laboratory based. These data are also often sufficient to verify food safety management systems.

Rapid tests and liquid chromatography methods for the rapid detection and quantification of toxins have been developed to HACCP purposes.

**Rapid tests based on immunoassay products**

Within the commercial environment it is critical for those purchasing raw materials to satisfy themselves that any delivery meets regulatory and specification requirements. Decisions to accept or reject deliveries usually need to be taken within short time periods. This requires the use of rapid reliable analytical methodology based in immunoassay products; furthermore, the methods need to be of minimal complexity as rapid test kits (Alldrick, 2014). Rapid test methods are increasingly being promoted as tools for food companies to validate and or verify the efficacy of their food safety management systems.

To enforce the various legal limits, it would be preferable to determine all mycotoxins by routine analysis in different types of matrices in one single extract. This would also be advantageous for HACCP control purposes. For this reason, a multi-method was developed with which 33 mycotoxins: Aflatoxins B1, B2, G1 and G2, ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin, HT-2 toxin, a-zearalenol, a-zearalanol, b-zearalanol, sterigmatocystin, cyclopiazonic acid, penicillic acid, fumonisins B1, B2 and B3, diacetoxyscirpenol, 3- and 15-acetyldeoxynivalenol, zearalanone, ergotamin, ergocornin, ergocristin, a-ergocryptin, citrinin, roquefortin C, fusarenone X, nivalenol, mycophenolic acid, alternariol and alternariol monomethyl ether. The multi-mycotoxin method has been proven by the detection of more than one mycotoxin in maize, buckwheat, figs and nuts. The LC–MS/MS technique has also been applied to baby food, which is subject to lower limits for aflatoxin B1 and ochratoxin A, ergot alkaloids in naturally contaminated rye and freeze-dried silage samples (Spanjer et al., 2008).

**Economic sectors sensitive to the contamination by mycotoxins**

It is included mycotoxins (Tables 1) and ochratoxin A (Table 2) in commodities and geographical distribution of HACCP studies.

<table>
<thead>
<tr>
<th>Table 1: Mycotoxins, commodities and geographical area of production</th>
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<tr>
<td>Aflatoxins</td>
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<td>Aflatoxins deoxynivalenol, ochratoxin A</td>
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<td>Aflatoxins Fumonisins</td>
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<td>Aflatoxins Deoxynivalenol Zearalenone</td>
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<td>Aflatoxins Cassava</td>
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<td>Aflatoxins B1,B2, G, G2</td>
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<td>Aflatoxin M1</td>
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**Sector of cereals**

China, one of the largest importers and exporters of food and animal feed, has implemented management strategies to protect consumers from mycotoxins and ensure safe trading of food and feed. The development and status of these management strategies are of interest to many stakeholders. There are 49 mycotoxin-related regulations in...
China; these include maximum levels for seven mycotoxins, standard methods for detecting 17 mycotoxins, and a code of practice for the prevention and reduction of mycotoxin contamination in cereals. Twelve mycotoxins found in daily food are covered by the Chinese contamination monitoring network. A new risk assessment organization is required to systematically implement and manage food safety risk assessments in China. In addition, self-control by companies and daily supervisions and inspections by government departments are quite effective in preventing the consumption of mycotoxin-contaminated food and feed (Zhang et al., 2014).

### Table 2: Ochratoxin, commodities and geographical area of study

<table>
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<tr>
<th>Pig Feed</th>
<th>Serbia</th>
<th>Milicevic et al., 2010</th>
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<tr>
<td>Cereals, grapes and coffee beans</td>
<td>England</td>
<td>Scudamore, 2005</td>
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<tr>
<td>Rice</td>
<td>Spain</td>
<td>Gonzalez et al., 2006</td>
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<tr>
<td>Cocoa</td>
<td>Venezuela</td>
<td>Lopez D’Olia et al., 2012</td>
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<tr>
<td>Coffee</td>
<td>Ecuador</td>
<td>Lopez-Garcia et al. 2008</td>
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<td>Red Paprika</td>
<td>Korea</td>
<td>Ahn et al., 2010</td>
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<td>Wine</td>
<td>Italy</td>
<td>Alonzo et al., 2008</td>
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<td>Wine</td>
<td>Italy</td>
<td>Breza et al., 2003</td>
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<tr>
<td>Wine</td>
<td>Spain</td>
<td>Martinez-Rodriguez &amp; Carrascosa AV, 2009</td>
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<tr>
<td>Olive</td>
<td>Turkey</td>
<td>Tokusoglu &amp; Bozoglu 2010</td>
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The agriculture sector plays an important role in the economy of any country, with livestock being an integral part. Feed production and costs are a major issue faced by international as well as local industries. As the same feed materials are shared by animals and humans alike, cereals like rice, wheat, corn and their by-products are used for the production of poultry feed. Due to this and the competitive nature of raw material pricing, typically lower quality ingredients are used in poultry feed. These materials may be stored for longer periods and due to poor storage conditions, contamination of these products can occur. Due to the ubiquitous nature of fungi, they contaminate these ingredients. Humid and hotter countries experience more fungal problems due to the environmental conditions and other aspects such as poor storage. The end result is the production of fungal metabolites within cereals and grains known as mycotoxins. Although many forms exist amongst mycotoxins, ochratoxins and aflatoxins are considered to be the most important. When more than one fungal contaminant is present, typically additive synergistic interactions are seen, increasing the toxicity within the feed. Determination of toxins can be done in tissues and feed samples by using different techniques such liquid chromatography and Hazard analysis critical control point (HACCP) should be referred to for the control of mycotoxins in the food chain. Due to increased consumer demand for poultry in almost all countries, this sector is gaining in importance. The poultry feed industry suffers the greatest economic losses compared to other animal species due to mycotoxins, as it heavily relies on cereals. Due to this, control strategies should be adapted to minimize the exposure of birds to mycotoxins. Control strategies are adapted at pre harvest, harvest and post-harvest levels. The hazard analysis critical control point (HACCP) system can be used to manage the risk of mycotoxins throughout the production (Abidin et al., 2011).

Approaches for the early detection and prevention strategies which have been employed in Serbia for the control of ochratoxigenic fungi and its metabolites in feed in the context of a HACCP framework are presented. The deoxynivalenol, ochratoxin A and zearealenol were detected, while aflatoxins were not present. Deoxynivalenol was detected in 10 samples in the concentration range 0.25-2.5 mg/kg. Ochratoxin A and zearealenol were detected in nine and eight samples, respectively, in the concentration range 0.057-0.27 and 0.2-5.0 mg/kg, respectively. These findings indicate that there may be a risk of animal exposure to mycotoxins through the consumption of mouldy infected feeds (Milicevic et al., 2010).

Feed control is important for the safety of foods of animal origin. Feed hazards include mycotoxins as Aflatoxins (Vlachou et al, 2004) responsible for food-borne diseases. A study was realized to evaluate the safety of samples consisting of compound feeds, feed materials and premixes, and provide information for the food chain. Aflatoxins were not found however the data meet demands of recent EU legislation for establishing specific criteria for feed manufacturers and fill gaps on the traceability and development of HACCP system in the animal production sector (Paramithiotis et al, 2009).

Maize (Zea mays L.) is one of the main cereals as a source of food, forage and processed products for industry. World production is around 800 million tons of maize because as a staple food it provides more than one third of the calories and proteins in some countries. Stored maize is a man-made ecosystem in which quality and nutritive changes occur because of interactions between physical, chemical and biological factors. Fungal spoilage and mycotoxin contamination are of major concern. Aspergillus and Fusarium species can infect maize pre-harvest, and mycotoxin contamination can increase if storage conditions are poorly managed. Prevention strategies to reduce the impact of mycotoxin in maize food and feed chains are based on using a hazard analysis critical control point systems (HACCP) approach. To reduce or prevent production of mycotoxins, drying should take place soon after harvest and as rapidly as feasible. The critical water content for safe storage corresponds to a water activity (aw) of about 0.7. Problems in maintaining an adequate low aw often occur in the tropics where high ambient humidity makes the control of commodity moisture difficult. Damaged grain is more prone to fungal invasion and, therefore, mycotoxin contamination. It is important to avoid damage before and during drying, and during storage. Drying maize on the cob before shelling is a very good practice. In storage, many insect species attack grain and the moisture that can accumulate from their activities provide ideal conditions for fungal activity. To avoid moisture and fungal contamination, it is essential that the numbers of insects in stored maize is kept to a minimum. It is possible to control fungal growth in stored commodities by controlled atmospheres, preservatives or natural inhibitors. Studies using antioxidants, essential oils under different conditions of aw, and temperature and controlled atmospheres have been evaluated as possible strategies for the reduction of fungal growth and mycotoxin as aflatoxins and fumonisins, in stored maize, but the cost of these treatments is likely to remain prohibitive for large-scale use (Chulze, 2010).

As maize quality before storage and storage conditions are of paramount importance, it was assessed microbial and mycotoxin contamination of 11 maize samples, comprising different combinations of healthy and damaged corn cobs. Tested parameters were bacterial, fungal and mycotoxin levels (deoxynivalenol, zearealenone 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 (Gaggiu et al., 2007).

To manage the hazards, aflatoxins associated with the production of an indigenous African fermented maize product, kenkey, HACCP was implemented at a semi-commercial kenkey production plant in Accra (Ghana). As prerequisite programme, the facility was upgraded and GMP implemented before HACCP. The effectiveness of GMP and HACCP was assessed by monitoring the environment and kenkey production, as well as the auditing and verification of HACCP (Amao-Awua et al, 2007). Ice-kenkey is a chilled cereal beverage sold as street food in some open markets in Ghana. It is produced by mashing and sweetening kenkey, a stiff dumpling produced from fermented maize, and sweetening kenkey, a stiff dumpling produced from fermented maize. The safety of street vended ice-kenkey was assessed by microbiological, elemental and myco-toxicological analysis of ice-kenkey and intermediary products obtained from 16 producers in four open markets in the Accra and Tema metropolis. The results of aflatoxin analysis showed high levels of aflatoxins in the ice-kenkey samples. The study showed the need to improve the safety of ice-kenkey production and vending in Accra. The use of maize and
groundnuts as raw materials exposed ice-kenkey to aflatoxin contamination and required strict control. The work enabled a training manual to be developed which has been used to training ice-kenkey producers in Accra (Atter et al, 2015).

Aflatoxin B1 (AFB1) is considered by the International Agency for Research on Cancer to be the most carcinogenic of all the aflatoxins. About 250,000 hepatocellular carcinoma related deaths are reported to occur annually in Africa due to aflatoxin ingestion where 80% of cases and deaths of liver cancer occur in Western and Central Africa. The carcinogens may be present in many raw and processed food and feed. Among these products, cereals and milk are very susceptible to mycotoxin contamination and are often used as ingredients in many infant food products. Samples dried lafun obtained from processors from two villages in Ogbonoso, Nigeria were analysed showing aflatoxins ranging from 1 to 1,600 µg/kg. The critical control points identified in the production were steeping, drying, packaging/storage, and the implementation of the corrective measures led to the production of laboratory-prepared lafun with improved microbiological safety (Lateef & Ojo, 2016).

Dried fruits
The groundnut is highly sensitive to contamination by toxigenic fungi in the field, before and after harvest, and during drying and storing. This explains the high prevalence of aflatoxins in groundnuts when compared to other agricultural products. Aspergillus flavus is actually abundant in the mycoflora extant in the soils in which the oleganous plant is planted. HACCP principles were employed to assess the food safety protocols needed to handle aflatoxin contamination in southern Brazil's groundnut based food industry and provide a model for the establishment of CCPs in groundnut-based food production in this geographical area in order to reduce or eliminate aflatoxin contamination. This methodology has been suggested by the Food and Agriculture Organization (FAO) of the United Nations. Reception of prime matter, groundnut storage, roasting and thermal treatment were the main CCPs identified. Critical factors were the determination of aflatoxin, moisture content and water activity (aw) during groundnut reception and storage, control of temperature, roasting time and thermal treatment in the groundnut-based food manufacturing. The critical limit for moisture was 8.2% and 0.6 was established for aw. In Brazil, the limit for aflatoxins B1, B2, G1 and G2 has been established at 20 ppb. Temperatures of 180°C/ 1h and 80°C/40 min were established for roasting and thermal treatment stages of groundnut-based food, respectively (Toregeani-Mendes et al, 2011).

Milk products
The quality of goat milk production of a goat cheese of Camembert type was studied. The process with the HACCP system implemented, enabled the production of a healthy and safe cheese with the well-known characteristics (Anka et al., 2008).

Cows’ milk can be contaminated by aflatoxin M-1. The measures to achieve this are part of a combined legislation for consumer's protection, and are mainly addressed to animal feed producers. Commercial feed manufacturers have to include a HACCP system, including monitoring of the hazards by a sufficiently equipped laboratory and adherence to the general rules by farmers of good agricultural practice including feeding and feed cultivation and storage. For aflatoxin M-1 the actual contamination is well below the threshold values. This demonstrates the possibility for an effective control of environmental contamination in the food chain from feed to milk (Bluethgen, 2007).

OTA in Food commodities
Whenever possible, preventing the formation of OTA in susceptible food commodities such as cereals, grapes and coffee beans should be a priority. Each product tends to host specific OTA producing fungi so that the environmental conditions and factors that produce the subsequent formation of OTA need to be understood. Codes of Practice for prevention and management of OTA are being developed and can be used in conjunction with a HACCP approach to protect the end consumer, in line with EU statutory limits. If prevention fails, an understanding of how concentrations change during the whole food chain may be useful in minimizing the concentrations reaching the consumer. OTA is quite heat stable under neutral conditions but may be partly broken down, e.g. in extrusion processing. In milling or other separation procedures, OTA will be concentrated or reduced in the resulting components. By products such as ‘cleanings’ or bran may contain high concentrations and are often used for animal feed. Introduction of guideline or statutory maximum concentrations for feed within the EU makes it essential that concentrations of OTA in such by-products are acceptable. Studies have emphasized that OTA in cereals is a storage problem that can be prevented if sound Codes of Practice are carefully followed and can be managed using a HACCP approach. Similar guidelines are being developed for other key food commodities such as grapes and coffee. Prevention should be the primary aim because OTA is quite heat stable and difficult to remove although it can be reduced at higher temperatures and during extrusion. OTA may increase during malting or may be reduced by enzymatic actions. In wheat and maize milling OTA is concentrated in cleanings, bran and other fractions derived from the seed coat. However these by-products are often used for animal feed and maximum permissible guideline limits for OTA are likely to operate within the EU from January 2006. In addition, barley and wheat may be grown on farm and used directly for animal feed. Bran based human foods present a particular problem in relationship to the 2-tier regulations of 5 and 3 µg kg⁻¹ for whole and finished cereal products because OTA is concentrated by a factor of approximately 3 on milling and is quite stable during most processes (Scudamore, 2005).

OTA has been related to cocoa beans. Although the shell separation from the nib has been reported as an effective measure to control this chemical hazard, ochratoxin prevalence study in cocoa beans produced in the country is recommended, and validate the winnowing step as well (Lopez D’Sola et al, 2012).

Coffee is an important export product of Ecuador. Producers are challenged by the implementation of regulatory limits for ochratoxin A. Ecuador has four coffee production areas and the potential for mycotoxin contamination varies due to different environmental conditions and cultural differences in harvesting, storage, processing and commercialization. The major contributors to contamination are the lack of selection during harvesting, delays in drying or rewetting, the lack of proper drying and storage conditions, the mixing of products with different levels of moisture, and the potential for cross-contamination. The long commercialization chain involves different intermediaries that use foreign materials to increase the weight of the product without consideration of quality. Preliminary results show that coffee managed using the models had a better quality, a lower contamination, a higher yield and better commercial value. The use of local resources and low-cost technology was important in demonstrating the practical approach (Lopez-Garcia et al, 2012).

Wines
The limits of presence of OTA in the wines have been established by the rule EEC 123/2005. With the purpose to prevent the formation of OTA in the wine (from the grape to the bottled product), a study has been conducted to compare the different techniques currently used, as the Good Agricultural Practices (BPA), the Good Practices of Manufacture (BPF) and the Good Practices of storage (BPS) with the system of HACCP, nowadays used only from the begin of the phase of processing of the agricultural products. It results that, concerning the BPAs, the application of the System HACCP on the primary production would bring neither some innovation nor qualitative improvement. The comparison among BPF, BPS and HACCP, confirms, instead, as the HACCP allows individualizing a series of additional critical points worth of attention during the process and maintenance of the grapes. Such result is in agreement with the new community rule 852/2004, which, also because of the difficulties in
the application of the HACCP to the primary production, confirms the necessity to follow, on the field, the hygienic respect of the sanitary measures. This involves the necessity of an increasing integration and cooperation among different professional workers (as hygienist and agronomist) (Alonzo et al., 2008).

Both monitoring programs and researches have been performed, aimed at individuating the status of contamination worldwide and CCP in the wine-making chain. All studies confirmed that red wines resulted in contamination more frequently and at higher levels than white wines. A study to carry out an automated LC method of analysis for the determination of OTA in wine samples, in order to process a high number of samples for HACCP purposes was performed. Method performance characteristics, such as repeatability, internal reproducibility, and accuracy, showed good performance and reliability of the method adequately matching with the criteria suggested by Committee European de Normalization (CEN) for the analysis of mycotoxins. The advantages coming out from this method are, therefore, the saving of time of analysis, the possibility to analyze large amounts of samples, the reduction of the employment of personnel, and the obtaining of all the requirements requested by the national legislation dealing with the official control of foodstuffs (Brera et al., 2003).

Red paprika

Large amount, 260,000 tons, of red paprika is consumed annually in Korea, where people prefer hot and pungent to sweet foods. Concern has recently grown among consumers over contamination of paprika powder by mycotoxins; contamination can occur at any stage from pre-harvest to drying, storage, grinding, and eventually transport to the retail market. A study was performed to investigate the current level of contamination of hot peppers by ochratoxin A and to identify the critical control points in the food chain. OTA content of 200 samples from various sources including supermarkets, an online shopping mall, small stakeholder mills, HACCP implemented factories, and an import company was measured. The data of two model factories that had adopted HACCP in different ways were compared in order to develop guidelines for alleviation of mitigation of the mycotoxin contamination. The dehydration and sterilization processes were selected as possible critical control points (CCPs) in the production chain (Ahn et al., 2010).

Conclusions

The best way to protect consumers against toxic effects of mycotoxins from food commodities is to apply the HACCP system for production and storage.

Several food commodities and feed industries are mainly interested in control mycotoxins as cereals, grapes, coffee, nuts, milks and wine manufacturers.

Aflatoxins and ochratoxins are the most determined mycotoxins for controlling the quality of the products by rapid tests and liquid chromatography methods.

Despite many years of research and the introduction of good practices in the food production, storage and distribution chain, mycotoxins are still a concern of food safety.

Conflict of interest

The authors report no conflict of interest.

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