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The Effect of Rapid Detection Methods on the Minimization and Prevention of the Risk of Contamination with Aflatoxins in Peanut Products

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Abstract

The methods of analysis and control of aflatoxins in peanuts pursue three key objectives: prevent the entry of contaminated peanuts into ready-to-eat products where they are used as an ingredient; prevent and minimize the risk of cross-contamination from contaminated peanuts to fit-for-use raw materials; perform an appropriate incoming inspection through rapid analysis methods for real-time detection of the absence of or the degree of contamination with aflatoxins. The aim of this study was to analyze the effect of rapid detection methods on the minimization and prevention of the risk of contamination with aflatoxins during the incoming inspection in industries using peanut products in the composition of the finished products. The methods of detection of aflatoxins in peanut products are: Mass Spectrometry combined with High - Performance Liquid Chromatography (HPLC), the internal methodology VAL 92:2010 developed by an accredited laboratory and immunochromatographic rapid tests.

Index terms— mycotoxins, aflatoxins, cross-contamination.

1 Introduction

eanuts are the raw material for the production of peanut butter, paste and oil, which are used as ingredients in various finished products such as biscuits, wafers and other confectionery products (Singh & Singh, 1991). As a good source of protein, peanuts are part of the balanced diet of many consumers (King et al., 2008), but, unfortunately, they are highly Author ?: Naval Academy "N. Y. Vaptsarov", Varna, Bulgaria, Varna 9026, V. Drumev St. ? 73. e-mails: marieta.stefanova@gmail.com, m.stefanova@nvna.eu Author ?: University of Economics -Varna, 77, Kniaz Boris I Blvd., 9002 Varna, Bulgaria. e-mail: spashova@ue-varna.bg susceptible to contamination by mycotoxins (Cotty & Jaime-Garcia, 2007). Their high nutritional value creates a favorable environment for developing and potential contamination by aflatoxins (De Oliveira & Corassin, 2014). Aflatoxins have been proven toxic and carcinogenic and likely to increase the frequency of mutations above the natural level (Creppy, 2002). In subtropical areas where temperatures and humidity are optimal for the growth of molds and the production of toxins (Gourama & Bullerman, 1995), such toxins contaminate the raw materials used in the production of cereal-based foods such as peanuts, rice, corn, etc. Aflatoxins can be effectively removed from the contaminated raw materials by physical, chemical and biological methods (Bata & Lásztity, 1999), each of which has its advantages and disadvantages. This requires taking effective control measures to reduce exposure (Goldblatt, 2012) and ensure compliance with the statutory requirements for the maximum level of aflatoxins in terms of food safety. The legal requirements for the maximum level for aflatoxins (aflatoxins B1, B2, G1, G2 and M1) are defined in Commission Regulation (EC) No. 1881/2006 (Commission Regulation (EC), 2006). The recommendation of the European Parliament is that the Regulation determines a level for aflatoxins in foodstuffs that is as low as reasonably achievable. The maximum levels are shown in Table 1. To assist the competent authorities in the official control of aflatoxin contamination, a "Guidance document for competent authorities for the control of compliance with EU legislation on aflatoxins" has been elaborated. In recent years, the European Food Safety Authority (EFSA) has adopted and published several scientific opinions on aflatoxins:

? September 1994, on the toxicological safety of aflatoxins B1, B2, G1, G2 and M1 (EFSA Panel, 2013); ? February 2004, on aflatoxins B1 as undesirable substances in animal feed (EFSA Panel, 2004); ? January 2018, on the potential increase of consumer health risk by a possible increase of the maximum levels for 'aflatoxin total' from 4 to 10 ?g/kg in peanuts and processed products thereof intended for direct human consumption or use as an ingredient in foodstuffs (EFSA Panel, 2018)

In recent years researchers have explored various methods for identification of aflatoxins in peanut products, with particular attention to the following critical factors for the reliability of the analysis: accuracy of the sampling methods, needed due to the heterogeneous distribution of aflatoxins in the peanut batches; the high quality of the analyses performed, and reliability of the results obtained by the different methods of analysis. The provisions relating to methods of sampling for mycotoxins, including aflatoxins, are laid down in Commission Regulation (EC) No. 401/2006.

The role of the rapid analysis methods during incoming inspection aimed at prevention and management of the risk of cross-contamination has not been sufficiently analyzed.

The aim of this study was to analyze the effect of rapid detection methods on the minimization and prevention of the risk of contamination with aflatoxins during an incoming inspection in industries using peanut products in the composition of the finished products.

2 II.

3 Methods

4 Materials.

To perform an analysis by the HPLC method, we tested three samples of peanuts of 500g each taken from batch C 23/0817 in an accredited laboratory. For the purposes of immunochromatographic rapid tests, we used six samples of peanuts of 500g each taken from batch C 23/0817.

Methods. Mass Spectrometry combined with High-Performance Liquid Chromatography (HPLC) was conducted in an accredited laboratory. A standardized method was implemented: based on the recommendations in ISO 16050:2003, an internal laboratory methodology VAL 92:2010 was developed to detect aflatoxins in cereals, nuts and derived products. The limit of quantification of aflatoxin B1 and aflatoxin total B1, B2, G1 and G2 was 8 ?g/kg. Immunochromatographic rapid tests. The sample was taken by the established sampling techniques and an extraction solution was prepared from the homogenized and finely ground peanuts 50 ± 0.2 g and 100 mL solution of 70% methanol / 30% distilled water. After preparing the mixture, filtering was carried out, using 2 to 3 mL of the extract for analysis. Using a pipettor, we pipetted portions of the six solutions (200 ?L) into six different vessels and put into the test strips for aflatoxins. After three minutes, the test strips were removed and the results were read by visual observation. Interpretation of results was made as follows: samples where the test strip showed two lines were reported as negative (less than 20 ppb aflatoxin) and samples where the test strip showed only one line was deemed positive (20 or more ppb aflatoxin). Where the visual check did not establish any line appearing in the control zone, the test was deemed invalid and retesting was made with another test strip.

5 III.

6 Results and Discussion

Several factors for aflatoxin contamination have been identified. The factor with the greatest weight is contamination occurring before harvest (Parmar et al., 1997). The treatment of peanuts reduces the formation of aflatoxins (Torres et al., 2014;Dorner, 2008) and the need for further corrective action. Although treatment is not always possible, prevention of contamination is the most effective method to combat all contaminants in foodstuffs. Upon receipt in the confectionery factory, peanuts must be checked under the procedures for incoming inspection. The recommended practice at this stage is to establish through documents control the origin of each batch of peanuts. Before performing an incoming inspection, it is necessary to establish that good hygiene practices were followed, especially during transportation, where contamination also can take place. The criteria for incoming inspection should be laid down in the specifications coordinated with the manufacturer of peanuts and peanut products intended for processing and use in the product. The specifications should include the maximum levels for aflatoxins and the respective methods and procedures of analysis and sampling. During the incoming inspection, it must be established that the supplied peanuts have no visible signs of deterioration; they are not musty or moldy and have not been infested by insects or rodents. The development of visible must or presence of mold eliminates the need for further analysis -the received batch must be isolated and rejected.

Under the existing legislation on food safety, food manufacturers must carry out control of all raw materials and ingredients under "Steps prior to hazard analysis" of the HACCP plan. The control of incoming raw materials should be documented in an Incoming Inspection Record. Raw materials must be inspected by personnel trained for carrying out such inspections. The preliminary control should include:

? Control for a sanitary condition of the vehicle used to supply the raw materials; ? Check the integrity of the packaging of the raw materials; ? Check for visible signs of pest infestation;

? Check the accompanying documents, establishing the date of manufacture, batch date and the minimum durability period or best before date; ? Check the temperature conditions during delivery, if the raw materials are supplied under controlled conditions, where relevant. ? Check that the raw materials comply with the agreed specification for delivery.

? Check the condition of the used for loading and transportation of the raw materials. The first stage of control of the supplied raw materials should include:

? Check that the quantity supplied corresponds to that indicated in the accompanying documents and agreed in contracts with the supplier;

? Documents control for compliance with what is indicated in the certificates of quality and safety of the shipment, including control of the origin of the raw material; ? Financial control for compliance of the price indicated in the accompanying documents with the agreed price of delivery; ? Control of the labeling and marking of the supplied raw material, to establish compliance with the contractual specification for the type of product, ingredients, storage conditions under temperature control, indications for specific uses.

The second stage of control includes carrying out laboratory tests. At this stage, the necessary analysis is conducted by microbiological and Physico-chemical indicators. The high risk of food contamination with mycotoxins requires an analysis to confirm the absence of aflatoxins in the supplied peanuts and peanut products. During the sampling for analysis, it should be borne in mind that the extreme heterogeneity of the possible contamination of peanuts with aflatoxins often leads to two types of errors. If the sample is smaller than the regulatory framework requires, this can lead to falsepositive results for mycotoxins and cause usable peanuts to be destroyed. There is also a second group of errors related to the occurrence of false -negative results for contaminated batches, which the laboratory testing designates as compliant. To avoid these types of errors, the sampling procedures should be followed very accurately, which is not always possible due to the lack of highly qualified personnel in production companies engaged in carrying out analyses. In addition, the sampling for analysis should also be in line with several economic factors related to the limited budget of the mycotoxin testing program, including the cost of sampling and sample preparation, cost of analysis and cost of sending the samples to an accredited laboratory where the actual analysis will be performed. It has been established that the most effective method for the quantitative determination of mycotoxins is high -performance liquid chromatography with mass spectroscopy, by which it is possible to simultaneously detect several types of mycotoxins in one sample. Although this method has its undeniable advantages and provides high precision, reliability and reproducibility of the results obtained, its application during incoming inspection of raw materials is extremely limited. Its shortcomings include high costs for carrying out analyses of multiple batches, the time required for transportation of the sample to an accredited laboratory, the carrying out of the actual analysis and the interpretation of the results, as the batch cannot be accepted before completion of all stages of the analysis.

These shortcomings, combined with the heterogeneity of the samples tested and the need for reliable methods for real-time detection of mycotoxins, lead to challenges for applying conventional and established methods of analysis and the implementation of ELISA-based methods of incoming inspection. Experience shows that the most common qualitative methods of incoming inspection are immunochromatographic rapid tests for mycotoxin analysis.

For this study, two types of analysis were carried out on the same batch of peanuts, C 23/0817, subjected to an incoming inspection. The results are shown in Table 2. The analyses carried out by both methods did not reveal the presence of mycotoxins in the tested sample from peanuts batch C 23/0817. This incoming inspection allows the batch to be accepted. The time spent for analysis by the method of HPLC was 48 hours, and that for analysis through immunochromatographic rapid tests was 30 minutes (including the time for preparation of the sample for analysis). From an economic perspective, the traditional analysis method required several times higher costs than the rapid tests for analysis.

In case of positive results in the incoming inspection and deviation from the specification, the controller of the batch must dispatch the peanuts or peanut products for storage. This would require that the batch be isolated from the usable raw materials and stored separately until a decision is taken to submit a claim to the supplier.

7 IV.

8 Conclusions

Mycotoxins, and in particular aflatoxins, have been proven to be toxic and carcinogenic even at very low concentrations, which requires sensitive and reliable methods of detection. The carrying out of analyses upon the incoming inspection of peanuts and peanut products is critical to verify their compliance with the safety requirements. The general analysis of mycotoxins by the HPLC method, performed in an accredited laboratory, provides reliable and accurate results, but are expensive and takes too much time to complete. This requires the introduction of rapid qualitative analysis tests that do not enable quantification of the test indicator but allow for real-time results of the analysis and a timely disposition of raw materials which do not meet the regulatory requirements for the presence of mycotoxins. We have concluded that producers who use peanuts as an ingredient in readyto-eat foodstuffs should implement rapid tests for the analysis of mycotoxins to increase efficiency and prevent cross-contamination. In addition, it is necessary to pay due attention to the creation of appropriate

162 conditions for the processing and storage of peanutbased finished and semi-finished products to prevent the development of mycotoxins in these materials.

1

Foodstuff	Maximum levels (?g/kg) B1	Sum of B1, B2, G1 and G2	M1
Peanuts and other oilseeds to be subjected to sorting or other physical treatment, before human consumption or use as an ingredient in foodstuffs, with the exception	8.0	15.0	-
of peanuts and other oilseeds to be subjected to grinding for the production of refined vegetable oil			
Peanuts and other oilseeds and processed products thereof intended for direct human consumption or use as an ingredient in foodstuffs, with the exception of	2.0	4.0	-
crude vegetable oils intended for refining and refined vegetable oils			

Figure 1: Table 1 :

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Sample No.	Mycotoxins	HPLC Test results (?g/kg) (value and uncertainty)	Immunochromatographic rapid tests Test results Qualitative analysis
Sample 1	Aflatoxin B1 Total aflatoxins B1, B2, G1 and G2	0.25 ± 20 ref % <1.0	Negative result -
Sample 2	Aflatoxin B1 Total aflatoxins B1, B2, G1 and G2	0.25 ± 20 ref % <1.0	Negative result
Sample 3	Aflatoxin B1 Total aflatoxins B1, B2, G1 and G2,	0.30 ± 20 ref % <1.0	Negative result
Sample 4	Total aflatoxins B1, B2, G1 and G2	not tested	Negative result
Sample 5	Total aflatoxins B1, B2, G1 and G2	not tested	Negative result
Sample 6	Total aflatoxins B1, B2, G1 and G2	not tested	Negative result

Figure 2: Table 2 :

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