

Key Words: aflatoxins B1; ELISA; mycotoxins; official control; peanut; total aflatoxins

Introduction

Aflatoxins are mycotoxins produced by two species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus* which are found in areas with hot and humid climates. They can enter the food chain as a result of infection of crops before or after harvest and are typically found in foods such as cereals, peanuts, dried fruits, nuts and spices. Aflatoxins include aflatoxins B1, B2, G1, G2 and M1. The most common in food is aflatoxins B1 and it is among the most potent genotoxic and carcinogenic aflatoxins. Because the toxicity of aflatoxins, exposure through food should be kept as low as possible.

Temperature and humidity are important parameters for the growth of fungi and because of that climate changes can have a great impact on the presence of mycotoxins in food.

The aim of the work is to show the presence of aflatoxins in peanuts (*Arachis hypogaea*) and peanuts products during official controls in the period from 2017 to 2022.

Methods

For the detection of aflatoxins the enzyme immunoassay (ELISA) is used. The immunoenzymatic ELISA test is a biochemical technique used to determine the presence of antigens (mycotoxins) in the analyzed sample. Specific antibodies are attached to the walls of the microtiter plate wells. By adding samples or standard solutions, the mycotoxin present will bind to specific antibodies on the walls of the wells. The bound antigen will be proven by adding another specific antibody to which peroxidase is bound (enzyme conjugate). Any unbound enzyme conjugate is removed by washing. Enzyme substrate and chromogen are then added to the wells. The bound enzyme conjugate converts the colorless chromogen into a blue colored product. The addition of the stop reagent leads to a change in color from blue to yellow. Color intensity is measured on a photometer at 450 nm.

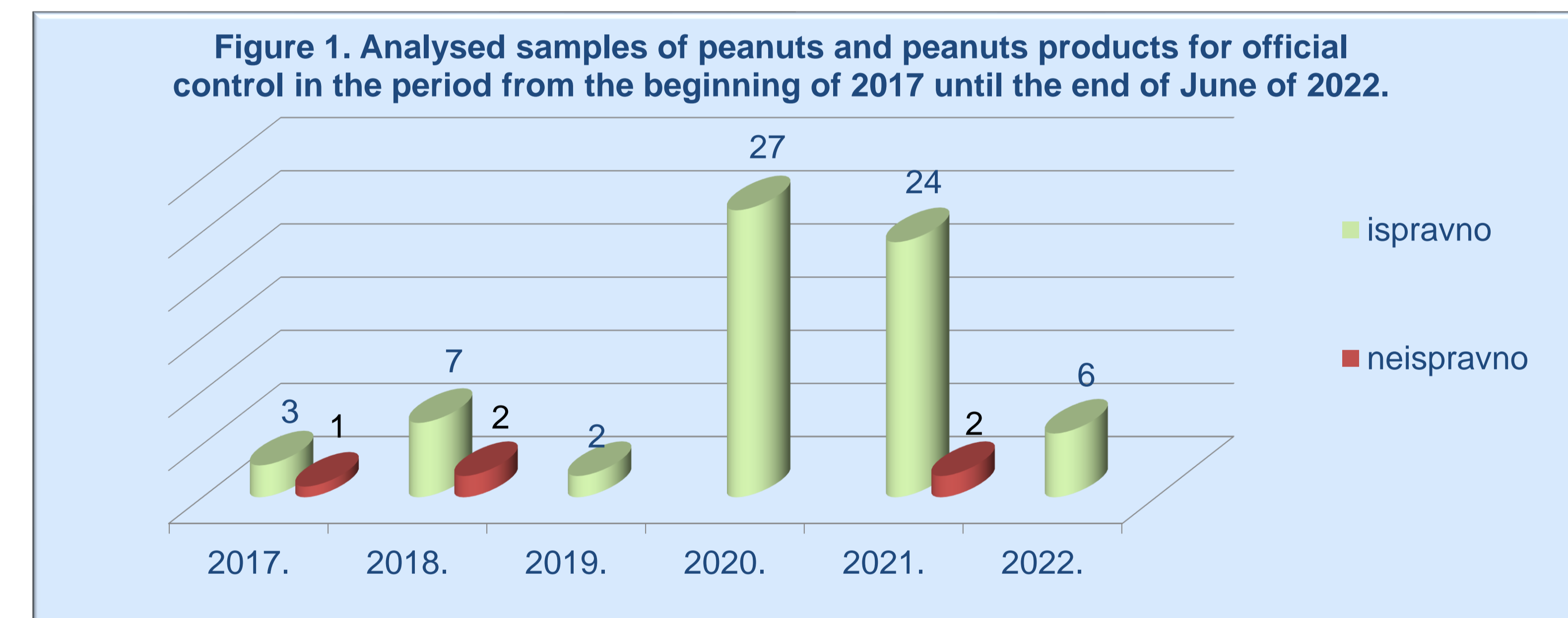
The RomerLabs "Aflatoxin B1 Assay" kit was used to determine aflatoxin B1 in the concentration range 2 - 50 µg/kg, and for determination of total aflatoxins the RomerLabs "Total Aflatoxin Assay" kit was used in the concentration range 1-20 µg/kg. TomerLabs kits contain all the necessary reagents and standards for aflatoxin analysis. Both methods for aflatoxin determination are accredited.

Results

Between the beginning of 2017 and the first six months of 2022 year 74 samples of peanuts and peanuts products were analysed for official control in the laboratory of the Department of food control of Teaching Institute of Public Health. There were imported samples of peanuts and peanuts products and the origin country was China. The sample was raw peanuts in the shell, raw peanut kernels or peanut butter. There were homogenised before analyse according to the Regulation No. 401/2006. Of the total 74 samples, 5 samples were positive for the presence of aflatoxins and not in accordance with Regulation No.1881/2006, which prescribes the maximum levels for aflatoxins. The maximum level of total aflatoxins for peanuts to be subjected to sorting, or other physical treatment, before human consumption is 15 µg/kg and for aflatoxin B1 is 8 µg/kg. For peanuts for direct human consumption maximum permissible level for total aflatoxins is 4 µg/kg and for aflatoxin B1 is 2 µg/kg. The aflatoxin concentrations in samples that did not comply with the regulation ranged from 12,7 – 73,99 µg/kg for aflatoxin B1 and 17,2 – 116,62 µg/kg for total aflatoxins. The number of analysed samples and samples positive on aflatoxins are shown in the Table 1. and Figure 1.

Table 1. The number of analysed samples of peanuts and peanuts products for official control in the period from the beginning of 2017 until the end of June of 2022.

	Total samples	Samples positive on aflatoxins
2017.	4	1
2018.	9	2
2019.	2	0
2020.	27	0
2021.	26	2
2022.	6	0



Discussion

From the obtained results it is evident that there was a significant number of samples which were positive on aflatoxins (over 22 %) in 2017. and 2018. During the 2019. there were analysed only 2 samples but during the 2020 year 27 samples were analysed on aflatoxins and in these two years there were no positive samples. In 2021 we found again 2 positive samples but it was less than 10 % (7,7 %). When we look at the entire period of more than 5 years, there were 6.8 % of samples that did not comply with Regulation No. 1881/2006. and pose a risk to human health due to their high toxicity. Aflatoxin B1 was the major contributor to total aflatoxins level. The concentrations of aflatoxins were compared with the results of other investigations of peanuts from China and other different parts of the world. Peanuts in China are heavily contaminated with aflatoxins, which pose a threat to human health. Wu et al. investigated the aflatoxins contamination of peanuts in 4 regions of China in the period from 2010 to 2013 and in the region of Sichuan Province found very high concentration of aflatoxins especially aflatoxin B1 which in one sample reached the 1390 µg/kg. In provinces of East and South China under a subtropical temperature monsoon climate like Shaanxi, Qin et.al. in peanut determined 35,18 µg/kg of total aflatoxins. According to Wang and Liu (2006) there was 3.03 % peanut samples exceeding the China national and Codex tolerance limits. Ding et.al. (2012) found the concentration of aflatoxin B1 above the permitted value in China which is 20 µg/kg in 0,6-6 % of peanut samples in different production area of China in 2009 and 2010.

Conclusion

Considering the high toxicity and high risk for consumer health and the proportion of defective samples, and because of climate changes it is very important to control the presence of aflatoxins in peanuts and other nuts imported from third countries and coming to the EU market. Considering the high risk associated with peanuts from certain provinces of China with a subtropical climate and based on the results of research and a great number of RASFF notification related to aflatoxins, special attention should be paid to samples of peanuts from China.

References: Commission Regulation (EC) No. 1881/2006, Commission Regulation (EC) No 401/2006, Wu L.X., Ding X.X., Li P.W., Du X.H., Zhou H.Y., Bai Y.Z. & Zhang L.X. Aflatoxin contamination of peanuts at harvest in China from 2010 to 2013 and its relationship with climatic conditions, *Food Control* (2015); Ding X., Li P., Bai Y. & Zhou H. Aflatoxin B1 in post-harvest peanuts and dietary risk in China, *Food Control* (2012); Qin M., Liang J., Yang D., Yang X., Cao P., Wang X., Ma N. Spatial analysis dietary exposure of aflatoxins in peanuts and peanut oil in different areas of China. Article in press, *Food Research International*; National Food Safety Standard for Maximum Levels of Mycotoxins in Foods (GB 2761-2017), National Health Commission and the China Food and Drug Administration (CFDA, currently the State Administration of Market Regulation); https://www.foodnavigator.com/Article/2018/05/02/Chinese-controls-for-aflatoxin-in-peanuts-exported-to-Europe?utm_source=copyright&utm_medium=OnSite&utm_campaign=copyright